

REAADS®

IgG Anti-Beta 2 Glycoprotein I Semi-Quantitative Test Kit

For *In Vitro* Diagnostic Use

An enzyme-linked immunosorbent assay (ELISA) for the semi-quantitative determination of IgG anti-Beta 2 Glycoprotein I (β_2 GPI) antibodies in human serum or citrated plasma (3.2% sodium citrate).

INTENDED USE

For the detection and semi-quantitation of IgG anti- β_2 GPI antibodies in individuals with systemic lupus erythematosus (SLE) and lupus-like disorders (anti-phospholipid syndrome).

SUMMARY AND EXPLANATION OF THE ANTI-BETA 2 GLYCOPROTEIN I TEST

Anti-phospholipid antibodies are a heterogeneous group of immunoglobulins that bind to several anionic phospholipids, including cardiolipin and phosphatidylserine.^{1,2} High serum levels of anti-phospholipid antibodies are frequently detected in patients with autoimmune (e.g., SLE) and non-autoimmune diseases, as well as in apparently healthy individuals.^{3,4} These antibodies have been associated with an increased risk for recurrent arterial and venous thrombotic events, thrombocytopenia, and fetal loss. These manifestations are the main features of the anti-phospholipid syndrome (APS).^{5,6}

Most autoimmune anti-phospholipid antibodies require a serum cofactor (β_2 GPI) for optimal binding.⁷⁻¹⁰ It has been shown that many anti-phospholipid antibodies may react to a neoepitope formed on the β_2 GPI molecule by the interaction between the phospholipid and β_2 GPI.^{11,12} Most assays for anti-phospholipid antibodies contain bovine serum as the source of cofactor. More recently, it has been shown that the binding of β_2 GPI to the microwell surface may produce a neoepitope similar to that when combined with a phospholipid and the results with this system showed a good correlation with the anti-phospholipid syndrome.¹³⁻¹⁶ The serologic detection of anti- β_2 GPI antibodies provides enhanced clinical sensitivity for thrombosis. The REAADS Anti- β_2 GPI ELISA Test Kit uses the well known ELISA format to detect anti- β_2 GPI antibodies in human serum.

Patients with positive reactions to both anti-phospholipid and anti- β_2 GPI assays were more likely to have clinical complications than those positive for only one. Higher prevalence and mean serum levels of IgG anti- β_2 GPI antibodies have been reported in autoimmune patients. In addition, anti- β_2 GPI antibodies in SLE patients correlated with clinical manifestations of anti-phospholipid syndrome.¹⁷

PRINCIPLE OF THE TEST

The test is performed as an indirect ELISA. Diluted serum or plasma samples, calibrator sera, and controls are incubated in microwells coated with purified human β_2 GPI. Incubation allows the anti- β_2 GPI antibodies present in the samples to react with the immobilized antigen. After the removal of unbound serum or plasma proteins by washing, antibodies specific for human IgG, labeled with horseradish peroxidase (HRP), are added forming complexes with the β_2 GPI bound antibodies. Following another washing step, the bound enzyme-antibody conjugate is assayed by the addition of a single solution containing tetramethylbenzidine (TMB) and hydrogen peroxide (H_2O_2) as the chromogenic substrate. Color develops in the wells at an intensity proportional to the serum concentration of anti- β_2 GPI antibodies.

Results are obtained by reading the O.D. (optical density or absorbance) of each well in a spectrophotometer. Calibrator sera are provided, with the IgG anti- β_2 GPI antibody concentrations expressed in G units. The user has the option of running either a single point calibrator or a four-point calibration curve. For single point calibration, dividing the concentration value of the calibrator sera by the O.D. value of the calibrator provides a conversion factor. The O.D. values of the other samples are multiplied by the conversion factor to obtain IgG anti- β_2 GPI antibody concentrations in G units. For multipoint calibration, perform a linear regression analysis with calibrator values against calibrator O.D.s. Controls and patient results are determined from the calibration curve. These units are traceable to available reference preparations.

REAGENTS

Store at 2 - 8°C. Do Not Freeze.

Each REAADS IgG Anti- β_2 GPI 96-Microwell Test Kit contains the following reagents (**volumes may vary depending on the kit size and configuration**):

- 12 x 8 stabilized β_2 GPI (from human serum) coated microwells with frame.
- 60 mL Sample Diluent IV (blue-green solution).
- 3 vials (0.250 mL) IgG β_2 GPI Calibrator Serum* (1-high, 2-moderate, 3-low)(human); see vial label for antibody concentration in G units. Calibrator 3 should be used when performing single point calibration.
- 0.250 mL IgG β_2 GPI Positive Control Serum* (human); see vial label for expected G unit range.
- 0.250 mL Normal Control Serum* (human); see vial label for expected G unit range.
- 15 mL anti-human IgG (goat) HRP-Conjugated Antibody Solution (blue solution).
- 15 mL One Component Substrate Solution (TMB and H₂O₂); ready to use.
- 15 mL Stopping Solution (0.36 N sulfuric acid).
- 2 bottles (30 mL) Wash Concentrate (33X PBS/Tween).

***CAUTION: Contains sodium azide**

WARNINGS AND PRECAUTIONS

For *In Vitro* Diagnostic Use

1. Human source material used to prepare the calibrators and controls included in this kit has been tested and shown to be negative to HBsAg, HCV, and HIV 1 & 2 by FDA required tests. However, all human blood derivatives, including patient samples, should be handled as potentially infectious material.
2. Do not pipette by mouth.
3. Do not smoke, eat, or drink in areas where specimens or kit reagents are handled.
4. Wear disposable gloves while handling kit reagents and wash hands thoroughly afterwards.
5. Certain components of this product contain sodium azide as a preservative. Sodium azide has been reported to form lead and copper azides when left in contact with these metals. These metal azides are explosive. Any solutions containing azide must be thoroughly flushed with copious amounts of water to prevent the build-up of explosive metal azides in the plumbing system.
6. One Component Substrate Solution can cause irritation to the eyes and skin. Use gloves when handling substrate and wash thoroughly after handling. Keep reagent away from ignition sources. Avoid contact with oxidizing agents.
7. Certain components are labeled with one or more of the following: Harmful if swallowed (R22). Avoid contact with skin and eyes (S24/25). In case of contact with eyes, rinse immediately with plenty of water and seek medical advice (S26). Wear suitable protective clothing and gloves (S36/37). Keep away from sources of ignition—no smoking (S16).

SPECIMEN COLLECTION AND PREPARATION

Serum or citrated plasma (3.2% sodium citrate) should be used as the sample matrix.

Blood should be collected by venipuncture and the serum separated from the cells by centrifugation after clot formation. If not tested immediately, the specimens should be stored at 2 to 8°C. If specimens are to be stored for more than 72 hours, they should be frozen at -20°C or below. Avoid repeated freezing and thawing. Do not use hemolyzed, icteric, or lipemic serum or plasma as these conditions may cause aberrant results. Specimens containing visible particulate matter should be clarified by centrifugation before testing.

If citrated plasma is to be used, blood should be collected by venipuncture and the plasma separated from the cells immediately by centrifugation at 1500g for 10 minutes. The supernatant must be carefully removed after centrifugation to avoid contamination with platelets. Repeating the centrifugation and separation steps may be advisable to minimize platelet contamination. Lysed or aged platelets can lead to aberrant results. If not tested immediately, plasma samples should be stored as described for serum.

INSTRUCTIONS FOR USE

Materials Provided

REAADS Anti- β_2 GPI Test Kit; see "Reagents" section for a complete list.

Materials Required but not Supplied

- Reagent grade water to prepare PBS wash solution and to zero or blank the plate reader during the final assay step
- Graduated cylinders
- Precision pipettors capable of delivering between 5 μ L and 1000 μ L, with appropriate tips
- Miscellaneous glassware appropriate for handling small volumes
- Flasks or bottles, 1 liter
- Wash bottles, preferably with the tip partially cut back to provide a wide stream, or an automated or semi-automated washing system
- Disposable gloves
- Plate-reading spectrophotometer capable of reading absorbance at 450 nm (650 nm reference if dual beam)
- Multichannel pipettors capable of delivering to 8 wells simultaneously
- Microdilution tubes and a 96-well microdilution tube holder for sample dilutions and rapid delivery to microwell plate

Procedural Notes

1. Bring serum or plasma samples and kit reagents to room temperature and mix well before using; avoid foaming. Return all unused samples and reagents to refrigerated storage as soon as possible.
2. All dilutions of calibrators, controls, and test sera or plasma must be made just prior to use in the assay.
3. A single water blank well can be included in each plate with each run. No sample or kit reagents are to be added to this well. Instead, add 200 μ L of reagent grade water to the well immediately prior to reading the plate in the spectrophotometer. The plate reader should be programmed to "zero" or "blank" against air or a water well.
4. Good washing technique is critical for optimal performance of the assay. Adequate washing is best accomplished by directing a forceful stream of wash solution into the bottom of the microwells from a plastic squeeze bottle with a wide tip. Wash solution in the water blank well will not interfere with the procedure. An automated plate washing system can also be used.
5. **IMPORTANT:** Failure to adequately remove residual wash solution can cause inconsistent color development of the Substrate Solution.
6. Use a multichannel pipettor capable of delivering to 8 wells simultaneously when possible. This speeds the process and provides more uniform incubation and reaction times for all wells.
7. Carefully controlled timing of all steps is critical. All calibrators, controls, and samples must be added within a five minute period. Batch size of samples should not be larger than the amount that can be added within this time period.
8. For all incubations, the start of the incubation period begins with the completion of reagent or sample addition.
9. Addition of all samples and reagents should be performed at the same rate and in the same sequence.
10. Incubation temperatures above or below normal room temperature (18 to 26°C) may contribute to inaccurate results.
11. Avoid microbial and cross-contamination of reagents when opening and removing aliquots from the primary vials.
12. Do not use kit components beyond expiration date.
13. Do not use kit components from different kit lot numbers.

Reagent Preparation

Wash Solution (PBS/Tween): Measure 30 mL of Wash Concentrate and dilute to 1 liter with reagent grade water. The pH of the final solution should be 7.35 ± 0.1 . Store unused wash solution in the refrigerator at 2 - 8°C. Discard if the solution shows signs of microbial or cross-contamination.

Assay Procedure

1. The assay can be performed with a single point calibration (Calibrator 3) or a four-point calibration curve (Calibrators 1, 2, and 3 plus sample diluent/reagent blank as Calibrator 4 equal to 0 G units). A reagent blank control should also be run with the single point and multipoint calibration method. Sample Diluent without serum or plasma is added to the well. This well will be treated the same as a control or patient sample in subsequent assay steps.
2. Remove any microwell strips that will not be used from the frame and store them in the bag provided.
3. Prepare a 1:50 dilution of the calibrators, controls, and patient samples in sample diluent (blue-green solution); e.g., 10 μ L sample added to 490 μ L Sample Diluent equals a 1:50 sample dilution.

4. Add 100 µL of diluted calibrators (including the reagent blank/Calibrator 4), controls, and patient samples to the appropriate microwells.
5. Incubate 15 minutes at room temperature. After the incubation is complete, carefully invert the microwells and empty the sample fluid. Do not allow samples to contaminate other microwells.
6. Wash 4 times with wash solution. Each well should be filled with wash solution per wash. Invert microwells between each wash to empty fluid. Use a snapping motion of the wrist to shake the liquid from the wells. To retain microwell modules during washing, the frame must be squeezed at the top and bottom of the longest sides. Blot on absorbent paper to remove residual wash fluid. Do not allow wells to dry out between steps.
7. Add 100 µL anti-human IgG HRP-Conjugated Antibody Solution (blue) to the wells.
8. Incubate for 15 minutes at room temperature. After the incubation is complete, carefully invert the microwells and empty the conjugate solution.
9. Wash 4 times with wash solution, as in step 6. Use a snapping motion to drain the liquid and blot on absorbent towels after the final wash. Do not allow the wells to dry out.
10. Add 100 µL One Component Substrate to each well and incubate for 10 minutes at room temperature. Add substrate to the wells at a steady rate. Blue color will develop in wells with positive samples.
11. Add 100 µL Stopping Solution (0.36 N sulfuric acid) to each well to stop the enzyme reaction. Be sure to add the acid to the wells in the same order and at the same rate as the Substrate was added. Blue Substrate will turn yellow and colorless solution will remain colorless. Blank or zero the plate reader against an air or a water blank well. Read the O.D. of each well at 450 nm (and 650 nm reference if dual beam). The O.D. values should be measured within 5 minutes of the addition of the Stopping Solution.

Results

Single Point Calibration

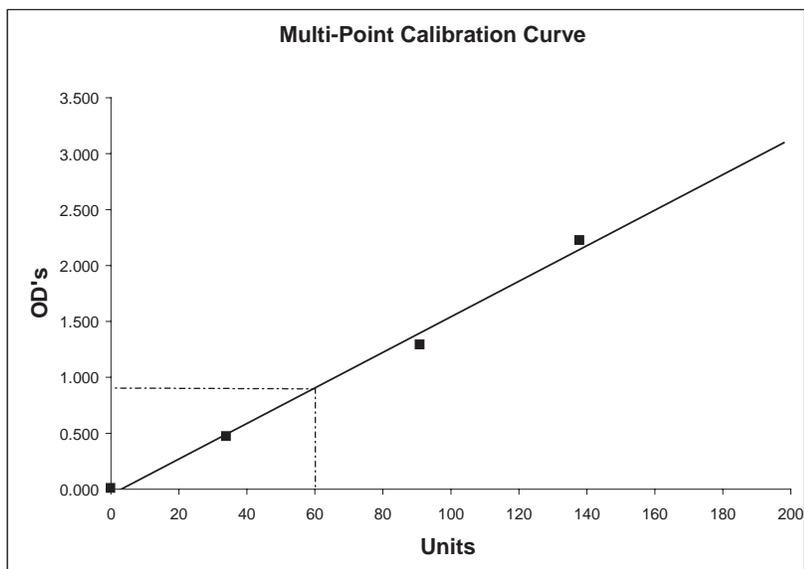
1. Calculate the mean O.D. values if duplicates of Calibrator 3, Controls and patient samples were performed.
2. Divide the concentration value of Calibrator 3 (printed on vial label) by the O.D. or mean O.D. value of the calibrator serum to obtain the conversion factor.
3. Multiply the O.D. or mean O.D. values for each of the controls and patient samples by the conversion factor to obtain an anti-β₂GPI antibody concentration value expressed in G units.

<p>Conversion Factor =</p> $\frac{\text{Anti-}\beta_2\text{GPI Concentration of Calibrator 3}}{\text{Absorbance Value of the Calibrator 3 (O.D.)}}$ <p>Anti-β₂GPI Concentration of Sample =</p> $\text{Conversion Factor} \times \text{Absorbance of the Sample (O.D.)}$

4. The Conversion Factor must be calculated for each assay run. Using a Conversion Factor from another assay will invalidate the results.

Multi-Point Curve Calibration

1. Calculate the mean O.D. values if duplicates of the calibrators, controls and patient samples were performed.
2. Perform linear regression analysis with the four calibrator values (See vial labels for G units. Calibrator 4 [sample diluent] is equal to 0 G units) against the mean O.D.s for each calibrator.
3. The calibrator curve can be plotted either automatically using a validated software program or manually with graph paper. It is recommended to use a zero intercept when generating the regression line to avoid negative values. If this option is not available, any negative values should be reported as zero units. When generating the curve manually, draw a best fit line through the plotted points using a zero intercept.
4. Determine the control and patient sample values from the calibrator curve.
5. Example of a multi-point curve calibration.



Using the example calibration curve provided, a specimen O.D. of 0.860 at 450 nm would correspond to a calculated value of 60 units. The calibration curve provided is an example only and should not be used to calculate patient results. A new calibration curve should be performed with every test run.

Quality Control

1. The O.D. value of Calibrator 3 should be at least 0.400 to assure that the kit is functioning properly. Calibrator 3 O.D. readings of less than 0.400 may indicate that the kit is no longer suitable for use.
2. The O.D. of Calibrator 4 or reagent blank should be less than 0.050 when the spectrophotometer has been blanked against air or a water well. Readings greater than 0.050 may indicate possible reagent contamination or inadequate plate washing.
3. The anti- β_2 GPI values obtained for the control sera should be within the ranges indicated on the vial labels. Occasional small deviations outside these ranges are acceptable.
4. O.D. values for duplicates of the controls or patient samples should be within 20% of the mean O.D. value for samples with absorbance readings greater than 0.200.
5. Each laboratory should periodically determine its own normal cut-off values for the appropriate population of patients.
6. Samples with anti- β_2 GPI values greater than 200 G units may be reported as "greater than 200 G units."
7. Assure that all quality control parameters have been met before reporting test results.

NORMAL RANGE

Serum samples from 120 healthy blood donors were tested for IgG anti- β_2 GPI antibodies. The following normal range was established:

- Less than 20 G units

PERFORMANCE CHARACTERISTICS

Clinical Specificity

Normal Samples:

Serum samples from 121 healthy blood donors were assayed for the presence of IgG anti- β_2 GPI antibodies. Using the establish cutoff value of 20 G units, this normal population demonstrated 100% specificity (mean value = 2.1 G units).

Serum samples from 41 infectious disease (syphilis), 42 progressive systemic sclerosis (PSS), and 42 rheumatoid arthritis (RA) patients were assayed for the presence of IgG anti- β_2 GPI antibodies. These patient groups demonstrated similar results compared to the healthy blood donor population (mean values = 3.9, 2.1, and 2.0 G units respectively). Results of these groups along with the healthy blood donors are summarized in the table below.

	Healthy	Infectious (syphilis)	PSS	RA
# of Samples (n)	121	41	42	42
Mean (G units)	2.1	3.9	2.1	2.0
Standard Deviation	1.1	10.3	3.9	2.2
% Negative	100%	97.5%	97.6%	100%

Clinical Sensitivity

Systemic Lupus Erythematosus (SLE):

Serum samples from 40 unselected (consecutive) patients with SLE were tested with the kit. Nine of the samples (sensitivity of 22.5%) were positive for IgG anti- β_2 GPI antibodies (mean value = 24.5 G units). A good correlation was found between IgG anti- β_2 GPI with both IgG anti-phosphatidylserine ($r = 0.928$) and IgG anti-cardiolipin ($r = 0.864$) antibody levels in this group.

Serum samples from 12 selected female patients with SLE who had a clinical history of thrombosis, thrombocytopenia, or recurrent fetal loss were evaluated for IgG anti- β_2 GPI antibodies. Seven of the samples (sensitivity of 58%) were positive in this population (mean value = 69 G units).

SLE Controls:

Serum samples from six selected female patients with SLE who had a history of thrombocytopenia (no thrombosis) were tested for IgG anti- β_2 GPI antibodies. Only one sample (17%) tested weak positive (36 G units), with a mean value of 12.7 G units for this group.

Serum samples from 10 selected female patients with SLE who were known not to have had thrombotic episodes, nor any other feature of the anti-phospholipid syndrome, were tested in the assay. Two of the samples (20%) were weak positive for IgG anti- β_2 GPI antibodies, with a mean value of 8.5 G units.

Primary Anti-phospholipid Syndrome (APS):

Serum samples from nine patients with the diagnosis of primary anti-phospholipid syndrome (APS) were tested on the REAADS IgG anti- β_2 GPI assay. Most, if not all, specimens were expected to be positive (above 20 G units) in this population. Eight of the nine samples resulted positive (88.9% sensitivity) with a mean value of 111 G units. A summary of sensitivity testing is presented below.

	Unselected SLE	Selected SLE No Thrombosis	Selected SLE Thrombosis	Primary APS
# of Samples (n)	40	16	12	9
Mean (G units)	24.5	10.1	69.0	111.0
Standard Deviation	49.6	12.7	66.2	55.2
% Positive	22.5%	18.8%	58.0%	88.9%

Technical Performance Comparison

Two disease populations (unselected SLE and primary APS) were tested on REAADS IgG anti- β_2 GPI ELISA assay and a predicate device to study the correlation between positive and negative results. The results are summarized in the table presented below.

REAADS IgG anti- β_2 GPI

Predicate Device IgG anti- β_2 GPI

	Negative	Positive		
Negative	32	8	Relative Sensitivity	100%
Positive	0	9	Relative Specificity	80%
			Agreement:	84%

Precision

Three samples with known G unit values (one low, one moderate, and one high) were assayed in 23 replicates on three different occasions. The mean intra-assay and inter-assay coefficients of variation (%CVs) are presented in the following table. The reported intra-assay coefficient of variation is the mean of the three separate intra-assay %CVs. Inter-assay %CV is the coefficient of variation obtained from three plates from one lot.

	Value Range	Mean Intra-assay %CV	Mean Inter-assay %CV
Low	(30 - 50 G units)	4.3%	4.0%
Moderate	(60 - 70 G units)	4.7%	3.5%
High	(>110 G units)	3.4%	1.7%

LIMITATIONS OF THE TEST

The anti- β_2 GPI antibody concentration values obtained from this assay are an aid to diagnosis only. Each physician must interpret these results in light of the patient's history, physical findings, and other diagnostic procedures. If clinical findings suggest the presence of anti-phospholipid antibodies and the patient is negative for anti- β_2 GPI antibodies, some investigators recommend testing for anti-cardiolipin antibodies, anti-phosphatidylserine antibodies, and the lupus anticoagulant to confirm the negative result. A patient may be considered positive for anti-phospholipid antibodies if one or all of the tests give positive results.

WARRANTY

This product is warranted to perform as described in this package insert. Corgenix, Inc. disclaims any implied warranty of merchantability or fitness for a particular use, and in no event shall Corgenix, Inc. be liable for consequential damage.

For Technical or Customer Service in the United States, phone 1-800-729-5661. Outside the United States, phone (303) 457-4345, fax (303) 457-4519, email: techsupport@corgenix.com or contact a Corgenix authorized distributor.

DEUTSCH

REAADS®

IgG Anti-Beta 2 Glycoprotein I Semi-Quantitative Test Kit

In-vitro-Diagnostikum

Ein enzymimmunologischer Test (ELISA) zur semiquantitativen Bestimmung von IgG-Anti-Beta 2 Glykoprotein I (β_2 GPI)-Antikörpern in Humanserum oder Zitratplasma (3,2 % Natriumzitat).

ANWENDUNGSBEREICH

Nachweis und semiquantitative Bestimmung von IgG Anti- β_2 GPI-Antikörpern bei Personen mit Lupus erythematosus (SLE) oder lupusartigen Erkrankungen (Antiphospholipid-Syndrom).

TESTPRINZIP

Der Test wird als indirekter ELISA durchgeführt. Proben verdünnten Serums oder Plasmas, Kalibratorseren und Kontrollen werden in Mikrovertiefungen inkubiert, die mit gereinigtem Human β_2 GPI beschichtet sind. Die Inkubation ermöglicht eine Reaktion der in den Proben enthaltenen Anti- β_2 GPI-Antikörper mit dem immobilisierten Antigen. Nach dem Auswaschen nicht gebundener Serum- oder Plasmaproteine werden mit Meerrettichperoxidase (HRP) markierte, für Human-IgG spezifische Antikörper zugefügt, die mit den an β_2 GPI gebundenen Antikörpern komplexieren. Nach einem weiteren Waschschrift wird das gebundene Enzym-Antikörper-Konjugat durch Zugabe einer Lösung, die Tetramethylbenzidin (TMB) und Wasserstoffperoxid enthält, (H_2O_2) angefärbt. In den Vertiefungen entsteht eine Färbung, deren Intensität in direkter Beziehung zur Anti- β_2 GPI-Antikörper-Serum-Konzentration steht.

Die Ergebnisse erhält man durch Ablesen der optischen Dichte (OD) bzw. Extinktion in allen Vertiefungen mit einem Spektrophotometer. Es werden Kalibrationsseren mitgeliefert, deren IgG Anti- β_2 GPI-Antikörper-Konzentration in G-Einheiten angegeben ist. Der Benutzer kann einen Einpunktkalibrator oder eine Vierpunkt-Kalibrierungskurve verwenden. Für die Einpunktkalibration wird die Konzentration der Kalibratorseren durch die optische Dichte (OD) des Kalibrators dividiert und ein Umrechnungsfaktor erhalten. Die OD-Werte der anderen Proben werden mit dem Umrechnungsfaktor multipliziert, um die Konzentration der IgG-Anti- β_2 GPI-Antikörper in G-Einheiten zu erhalten. Zur

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EC/REP Authorized Representative
(Bevollmächtigter)
(Représentant autorisé)
(Representante autorizado)
(Rappresentante autorizzato)

IVD In vitro diagnostic medical device
(In-vitro-Diagnostikum)
(Dispositif de diagnostic in vitro)
(Dispositivo médico para diagnóstico in vitro)
(Dispositivo medico-diagnostico in vitro)

LOT Batch
(Charge)
(Lot)
(Lote)
(Lotto)

 Expiry date
(Verfallsdatum)
(Date de péremption)
(Fecha de caducidad)
(Data di scadenza)

 Storage conditions
(Lagerbedingungen)
(Conditions de stockage)
(Condiciones de almacenamiento)
(Condizioni di conservazione)

Xn Harmful
(Gesundheitsschädlich)
(Nocif)
(Perjudicial)
(Nocivo)

Xi Irritant
(Reizend)
(Irritant)
(Irritante)
(Irritante)

 Biological Risk
(Biologisches Risiko)
(Risque biologique)
(Riesgo biológico)
(Rischio biologico)



Authorized Representative in the EU:

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Altenhofstraße 80
D-66386 St. Ingbert/Germany

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Anti-phospholipid antibodies are a heterogeneous group of immunoglobulins that bind to several anionic phospholipids, including cardiolipin and phosphatidylserine.^{1,2} High serum levels of anti-phospholipid antibodies are frequently detected in patients with autoimmune (e.g., SLE) and non-autoimmune diseases, as well as in apparently healthy individuals.^{3,4} These antibodies have been associated with an increased risk for recurrent arterial and venous thrombotic events, thrombocytopenia, and fetal loss. These manifestations are the main features of the anti-phospholipid syndrome (APS).^{5,6}

Most autoimmune anti-phospholipid antibodies require a serum cofactor (β_2 GPI) for optimal binding.⁷⁻¹⁰ It has been shown that many anti-phospholipid antibodies may react to a neoepitope formed on the β_2 GPI molecule by the interaction between the phospholipid and β_2 GPI.^{11,12} Most assays for anti-phospholipid antibodies contain bovine serum as the source of cofactor. More recently, it has been shown that the binding of β_2 GPI to the microwell surface may produce a neoepitope similar to that when combined with a phospholipid and the results with this system showed a good correlation with the anti-phospholipid syndrome.¹³⁻¹⁶ The serologic detection of anti- β_2 GPI antibodies provides enhanced clinical sensitivity for thrombosis. The REAADS Anti- β_2 GPI ELISA Test Kit uses the well known ELISA format to detect anti- β_2 GPI antibodies in human serum.

Patients with positive reactions to both anti-phospholipid and anti- β_2 GPI assays were more likely to have clinical complications than those positive for only one. Higher prevalence and mean serum levels of IgA anti- β_2 GPI antibodies have been reported in autoimmune patients. In addition, anti- β_2 GPI antibodies in SLE patients correlated with clinical manifestations of anti-phospholipid syndrome.¹⁷

PRINCIPLE OF THE TEST

The test is performed as an indirect ELISA. Diluted serum or plasma samples, calibrator sera, and controls are incubated in microwells coated with purified human β_2 GPI. Incubation allows the anti- β_2 GPI antibodies present in the samples to react with the immobilized antigen. After the removal of unbound serum or plasma proteins by washing, antibodies specific for human IgA, labeled with horseradish peroxidase (HRP), are added forming complexes with the β_2 GPI bound antibodies. Following another washing step, the bound enzyme-antibody conjugate is assayed by the addition of a single solution containing tetramethylbenzidine (TMB) and hydrogen peroxide (H_2O_2) as the chromogenic substrate. Color develops in the wells at an intensity proportional to the serum concentration of anti- β_2 GPI antibodies.

Results are obtained by reading the O.D. (optical density or absorbance) of each well in a spectrophotometer. Calibrator sera are provided, with the IgA anti- β_2 GPI antibody concentrations expressed in A units. The user has the option of running either a single point calibrator or a four-point calibration curve. For single point calibration, dividing the concentration value of the calibrator sera by the O.D. value of the calibrator provides a conversion factor. The O.D. values of the other samples are multiplied by the conversion factor to obtain IgA anti- β_2 GPI antibody concentrations in A units. For multipoint calibration, perform a linear regression analysis with calibrator values against calibrator O.D.s. Controls and patient results are determined from the calibration curve.

REAGENTS

Store at 2 - 8°C. Do Not Freeze.

Each REAADS IgA Anti- β_2 GPI 96-Microwell Test Kit contains the following reagents (**volumes may vary depending on the kit size and configuration**):

- 12 x 8 stabilized β_2 GPI (from human serum) coated microwells with frame.
- 60 mL Sample Diluent IV (blue-green solution).
- 3 vials (0.250 mL) IgA β_2 GPI Calibrator Serum* (1-high, 2-moderate, 3-low) (human); see vial label for antibody concentration in A units. Calibrator 3 should be used when performing single point calibration.
- 0.250 mL IgA β_2 GPI Positive Control Serum* (human); see vial label for expected A unit range.
- 0.250 mL Normal Control Serum* (human); see vial label for expected A unit range.
- 15 mL anti-human IgA (goat) HRP-Conjugated Antibody Solution (orange solution).
- 15 mL One Component Substrate Solution (TMB and H₂O₂); ready to use.
- 15 mL Stopping Solution (0.36 N sulfuric acid).
- 2 bottles (30 mL) Wash Concentrate (33X PBS/Tween).

***CAUTION: Contains sodium azide**

WARNINGS AND PRECAUTIONS

For *In Vitro* Diagnostic Use

1. Human source material used to prepare the calibrators and controls included in this kit has been tested and shown to be negative to HBsAg, HCV, and HIV 1 & 2 by FDA required tests. However, all human blood derivatives, including patient samples, should be handled as potentially infectious material.
2. Do not pipette by mouth.
3. Do not smoke, eat, or drink in areas where specimens or kit reagents are handled.
4. Wear disposable gloves while handling kit reagents and wash hands thoroughly afterwards.
5. Certain components of this product contain sodium azide as a preservative. Sodium azide has been reported to form lead and copper azides when left in contact with these metals. These metal azides are explosive. Any solutions containing azide must be thoroughly flushed with copious amounts of water to prevent the build-up of explosive metal azides in the plumbing system.
6. One Component Substrate Solution can cause irritation to the eyes and skin. Use gloves when handling substrate and wash thoroughly after handling. Keep reagent away from ignition sources. Avoid contact with oxidizing agents.
7. Certain components are labeled with one or more of the following: Harmful if swallowed (R22). Avoid contact with skin and eyes (S24/25). In case of contact with eyes, rinse immediately with plenty of water and seek medical advice (S26). Wear suitable protective clothing and gloves (S36/37). Keep away from sources of ignition—no smoking (S16).

SPECIMEN COLLECTION AND PREPARATION

Serum or citrated plasma (3.2% sodium citrate) should be used as the sample matrix. Blood should be collected by venipuncture and the serum separated from the cells by centrifugation after clot formation. If not tested immediately, the specimens should be stored at 2 to 8°C. If specimens are to be stored for more than 72 hours, they should be frozen at -20°C or below. Avoid repeated freezing and thawing. Do not use hemolyzed, icteric, or lipemic serum or plasma as these conditions may cause aberrant results. Specimens containing visible particulate matter should be clarified by centrifugation before testing.

If citrated plasma is to be used, blood should be collected by venipuncture and the plasma separated from the cells immediately by centrifugation at 1500g for 10 minutes. The supernatant must be carefully removed after centrifugation to avoid contamination with platelets. Repeating the centrifugation and separation steps may be advisable to minimize platelet contamination. Lysed or aged platelets can lead to aberrant results. If not tested immediately, plasma samples should be stored as described for serum.

INSTRUCTIONS FOR USE

Materials Provided

REAADS Anti- β_2 GPI Test Kit; see “Reagents” section for a complete list.

Materials Required but not Supplied

- Reagent grade water to prepare PBS wash solution and to zero or blank the plate reader during the final assay step
- Graduated cylinders
- Precision pipettors capable of delivering between 5 μ L and 1000 μ L, with appropriate tips
- Miscellaneous glassware appropriate for handling small volumes
- Flasks or bottles, 1 liter
- Wash bottles, preferably with the tip partially cut back to provide a wide stream, or an automated or semi-automated washing system
- Disposable gloves
- Plate-reading spectrophotometer capable of reading absorbance at 450 nm (650 nm reference if dual beam)
- Multichannel pipettors capable of delivering to 8 wells simultaneously
- Microdilution tubes and a 96-well microdilution tube holder for sample dilutions and rapid delivery to microwell plate

Procedural Notes

1. Bring serum or plasma samples and kit reagents to room temperature and mix well before using; avoid foaming. Return all unused samples and reagents to refrigerated storage as soon as possible.
2. All dilutions of calibrators, controls, and test sera or plasma must be made just prior to use in the assay.
3. A single water blank well can be included in each plate with each run. No sample or kit reagents are to be added to this well. Instead, add 200 μ L of reagent grade water to the well immediately prior to reading the plate in the spectrophotometer. The plate reader should be programmed to “zero” or “blank” against an air or a water well.
4. Good washing technique is critical for optimal performance of the assay. Adequate washing is best accomplished by directing a forceful stream of wash solution into the bottom of the microwells from a plastic squeeze bottle with a wide tip. Wash solution in the water blank well will not interfere with the procedure. An automated plate washing system can also be used.
5. **IMPORTANT:** Failure to adequately remove residual wash solution can cause inconsistent color development of the Substrate Solution.
6. Use a multichannel pipettor capable of delivering to 8 wells simultaneously when possible. This speeds the process and provides more uniform incubation and reaction times for all wells.
7. Carefully controlled timing of all steps is critical. All calibrators, controls, and samples must be added within a five minute period. Batch size of samples should not be larger than the amount that can be added within this time period.
8. For all incubations, the start of the incubation period begins with the completion of reagent or sample addition.
9. Addition of all samples and reagents should be performed at the same rate and in the same sequence.
10. Incubation temperatures above or below normal room temperature (18 to 26°C) may contribute to inaccurate results.
11. Avoid microbial and cross-contamination of reagents when opening and removing aliquots from the primary vials.
12. Do not use kit components beyond expiration date.
13. Do not use kit components from different kit lot numbers.

Reagent Preparation

Wash Solution (PBS/Tween): Measure 30 mL of Wash Concentrate and dilute to 1 liter with reagent grade water. The pH of the final solution should be 7.35 ± 0.1 . Store unused wash solution in the refrigerator at 2 - 8°C. Discard if the solution shows signs of microbial or cross-contamination.

Assay Procedure

1. The assay can be performed with a single point calibration (Calibrator 3) or a four-point calibration curve (Calibrators 1, 2, and 3 plus sample diluent/reagent blank as Calibrator 4 equal to 0 A units). A reagent blank control should also be run with the single point and multipoint calibration method. Sample Diluent without serum or plasma is added to the well. This well will be treated the same as a control or patient sample in subsequent assay steps.
2. Remove any microwell strips that will not be used from the frame and store them in the bag provided.
3. Prepare a 1:50 dilution of the calibrators, controls, and patient samples in sample diluent (blue-green solution); e.g., 10 μ L sample added to 490 μ L Sample Diluent equals a 1:50 sample dilution.
4. Add 100 μ L of diluted calibrators (including the reagent blank/Calibrator 4), controls, and patient samples to the appropriate microwells.
5. Incubate 15 minutes at room temperature. After the incubation is complete, carefully invert the microwells and empty the sample fluid. Do not allow samples to contaminate other microwells.
6. Wash 4 times with wash solution. Each well should be filled with wash solution per wash. Invert microwells between each wash to empty fluid. Use a snapping motion of the wrist to shake the liquid from the wells. To retain microwell modules during washing, the frame must be squeezed at the top and bottom of the longest sides. Blot on absorbent paper to remove residual wash fluid. Do not allow wells to dry out between steps.
7. Add 100 μ L anti-human IgA HRP-Conjugated Antibody Solution (orange) to the wells.
8. Incubate for 15 minutes at room temperature. After the incubation is complete, carefully invert the microwells and empty the conjugate solution.
9. Wash 4 times with wash solution, as in step 6. Use a snapping motion to drain the liquid, and blot on absorbent towels after the final wash. Do not allow the wells to dry out.
10. Add 100 μ L One Component Substrate to each well and incubate for 10 minutes at room temperature. Add substrate to the wells at a steady rate. Blue color will develop in wells with positive samples.
11. Add 100 μ L Stopping Solution (0.36 N sulfuric acid) to each well to stop the enzyme reaction. Be sure to add the acid to the wells in the same order and at the same rate as the Substrate was added. Blue Substrate will turn yellow and colorless solution will remain colorless. Blank or zero the plate reader against an air or a water blank well. Read the O.D. of each well at 450 nm (and 650 nm reference if dual beam). The O.D. values should be measured within 5 minutes of the addition of the Stopping Solution.

RESULTS

Single Point Calibration

1. Calculate the mean O.D. values if duplicates of Calibrator 3, Controls and patient samples were performed.
2. Divide the concentration value of Calibrator 3 (printed on vial label) by the O.D. or mean O.D. value of the calibrator serum to obtain the conversion factor.
3. Multiply the O.D. or mean O.D. values for each of the controls and patient samples by the conversion factor to obtain an anti- β_2 GPI antibody concentration value expressed in A units.

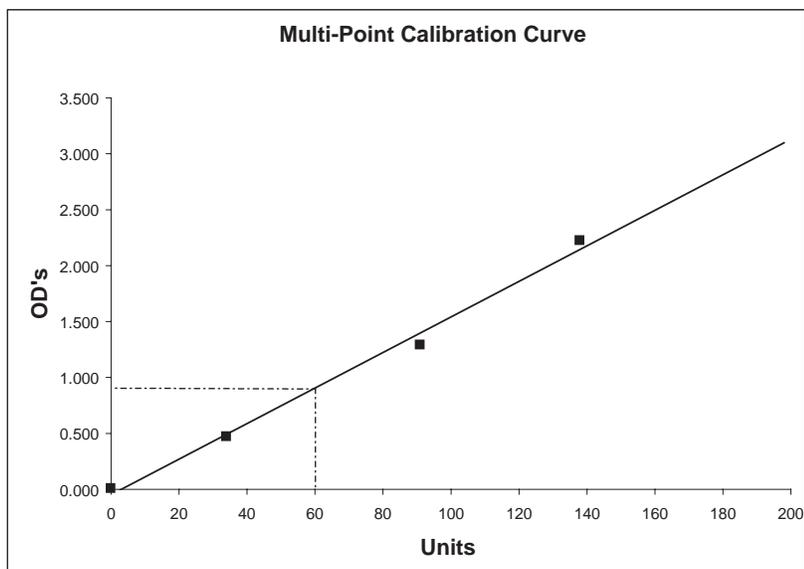
$$\text{Conversion Factor} = \frac{\text{Anti-}\beta_2\text{GPI Concentration of Calibrator 3}}{\text{Absorbance Value of the Calibrator 3 (O.D.)}}$$
$$\text{Anti-}\beta_2\text{GPI Concentration of Sample} = \text{Conversion Factor} \times \text{Absorbance of the Sample (O.D.)}$$

4. The Conversion Factor must be calculated for each assay run. Using a Conversion Factor from another assay will invalidate the results.

Multi-Point Curve Calibration

1. Calculate the mean O.D. values if duplicates of the calibrators, controls and patient samples were performed.
2. Perform linear regression analysis with the four calibrator values (See vial labels for A units. Calibrator 4 [sample diluent] is equal to 0 A units.) against the mean O.D.s for each calibrator.
3. The calibrator curve can be plotted either automatically using a validated software program or manually with graph paper. It is recommended to use a zero intercept when generating the regression line to avoid negative values. If this option is not available, any negative values should be reported as zero units. When generating the curve manually, draw a best fit line through the plotted points using a zero intercept.
4. Determine the control and patient sample values from the calibrator curve.

5. Example of a multi-point curve calibration.



Using the example calibration curve provided, a specimen O.D. of 0.860 at 450 nm would correspond to a calculated value of 60 units. The calibration curve provided is an example only and should not be used to calculate patient results. A new calibration curve should be performed with every test run.

Quality Control

1. The O.D. value of Calibrator 3 should be at least 0.400 to assure that the kit is functioning properly. Calibrator 3 O.D. readings of less than 0.400 may indicate that the kit is no longer suitable for use.
2. The O.D. of Calibrator 4 or reagent blank should be less than 0.100 when the spectrophotometer has been blanked against air or a water well. Readings greater than 0.100 may indicate possible reagent contamination or inadequate plate washing.
3. The anti-β₂GPI values obtained for the control sera should be within the ranges indicated on the vial labels. Occasional small deviations outside these ranges are acceptable.
4. O.D. values for duplicates of the controls or patient samples should be within 20% of the mean O.D. value for samples with absorbance readings greater than 0.200.
5. Each laboratory should periodically determine its own normal cut-off values for the appropriate population of patients.
6. Samples with anti-β₂GPI values greater than 200 A units may be reported as "greater than 200 A units."
7. Assure that all quality control parameters have been met before reporting test results.

NORMAL RANGE

Serum samples from 120 healthy blood donors were tested for IgA anti-β₂GPI antibodies. The following normal range was established:

- Less than 20 A units

PERFORMANCE CHARACTERISTICS

Clinical Specificity

Normal Samples:

Serum samples from 120 healthy blood donors were assayed for the presence of IgA anti-β₂GPI antibodies. Using the establish cutoff value of 20 A units, this normal population demonstrated 96% specificity (mean value = 6.9 A units).

Serum samples from 41 infectious disease (syphilis), 42 progressive systemic sclerosis (PSS), and 42 rheumatoid arthritis (RA) patients were assayed for the presence of IgA anti-β₂GPI antibodies. These patient groups demonstrated similar results compared to the healthy blood donor population (mean values = 9.9, 13.4, and 12.2 A units respectively). Results of these groups along with the healthy blood donors are summarized in the table below.

	Healthy	Infectious (syphilis)	PSS	RA
# of Samples (n)	120	41	42	42
Mean (A units)	6.9	9.9	13.4	12.2
Standard Deviation	5.7	6.5	6.5	10.4
% Negative	95.8%	95.1%	95.2%	83.3%

Clinical Sensitivity

Systemic Lupus Erythematosus (SLE):

Serum samples from 40 unselected (consecutive) patients with SLE were tested with the kit. Ten of the samples (sensitivity of 25%) were positive for IgA anti- β_2 GPI antibodies (mean value = 43 A units).

Serum samples from twelve selected female patients with SLE who had a clinical history of thrombosis, thrombocytopenia, or recurrent fetal loss were evaluated for IgA anti- β_2 GPI antibodies. Eight of the samples (sensitivity of 67%) were positive (mean value = 105 A units).

SLE Controls:

Serum samples from five selected female patients with SLE who had history of thrombocytopenia (no thrombosis) were tested for IgA anti- β_2 GPI antibodies. None tested positive, and the resulting mean value was 11 A units for this group.

Serum samples from nine selected female patients with SLE who were known not to have had thrombotic episodes, nor any other feature of the anti-phospholipid syndrome, were tested with this assay. One of the samples (11%) was strong positive (154 A units) for IgA anti- β_2 GPI antibodies. This group had a mean value of 22 A units.

Primary Anti-phospholipid Syndrome (APS):

Serum samples from nine patients with the diagnosis of primary anti-phospholipid syndrome (APS) were tested on the REAADS IgA anti- β_2 GPI assay. Most samples were expected to be positive (above 20 A units) in this population. Seven of the nine samples resulted positive (78% sensitivity) with a mean value of 132 A units. A summary of sensitivity testing is presented below.

	Unselected SLE	Selected SLE		Primary APS
		No Thrombosis	Thrombosis	
# of Samples (n)	40	14	12	9
Mean (A units)	42.6	18.3	105.6	132.4
Standard Deviation	72.5	39.3	97.5	89.3
% Positive	25.0%	7.1%	67%	77.8%

Technical Performance Comparison

Two disease populations (unselected SLE and primary APS) were tested on REAADS IgA anti- β_2 GPI ELISA assay and a predicate device to study the correlation between positive and negative results. The results are summarized in the table presented below.

		REAADS IgA anti-β_2GPI			
		Negative	Positive		
Predicate Device IgA anti-β_2GPI	Negative	30	6	Relative Sensitivity	85%
	Positive	2	11	Relative Specificity	83%
				Agreement:	84%

Precision

Three samples with known A unit values (one low, one moderate, and one high) were assayed in 23 replicates on three different occasions. The mean intra-assay and inter-assay coefficients of variation (%CVs) are presented in the table below. The reported intra-assay coefficient of variation is the mean of the three separate intra-assay %CVs. Inter-assay %CV is the coefficient of variation obtained from three plates from one lot.

Value Range	Mean Intra-assay %CV	Mean Inter-assay %CV
Low (27 - 37 A units)	4.7%	4.7%
Moderate (57 - 83 A units)	4.4%	3.7%
High (90 - 150 A units)	3.5%	3.7%

LIMITATIONS OF THE TEST

The anti- β_2 GPI antibody concentration values obtained from this assay are an aid to diagnosis only. Each physician must interpret these results in light of the patient's history, physical findings, and other diagnostic procedures. If clinical findings suggest the presence of anti-phospholipid antibodies and the patient is negative for anti- β_2 GPI antibodies, some investigators recommend testing for anti-cardiolipin antibodies, anti-phosphatidylserine antibodies, and the lupus anticoagulant to confirm the negative result. A patient may be considered positive for anti-phospholipid antibodies if one or all of the tests give positive results.

Warranty

This product is warranted to perform as described in this package insert. Corgenix, Inc. disclaims any implied warranty of merchantability or fitness for a particular use, and in no event shall Corgenix, Inc. be liable for consequential damage.

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DEUTSCH

REAADS®

IgA Anti-Beta 2 Glycoprotein I Semi-Quantitative Test Kit

In-vitro-Diagnostikum

Ein enzymimmunologischer Test (ELISA) zur semiquantitativen Bestimmung von IgA-Anti-Beta 2 Glykoprotein I (β_2 GPI)-Antikörpern in Humanserum oder Zitratplasma (3,2 % Natriumzitat).

ANWENDUNGSGEBIET

Nachweis und semiquantitative Bestimmung von IgA Anti- β_2 GPI-Antikörpern bei Personen mit Lupus erythematosus (SLE) oder lupusartigen Erkrankungen (Antiphospholipid-Syndrom).

TESTPRINZIP

Der Test wird als indirekter ELISA durchgeführt. Proben verdünnten Serums oder Plasmas, Kalibratorseren und Kontrollen werden in Mikrovertiefungen inkubiert, die mit gereinigtem Human β_2 GPI beschichtet sind. Die Inkubation ermöglicht eine Reaktion der in den Proben enthaltenen Anti- β_2 GPI-Antikörper mit dem immobilisierten Antigen. Nach dem Auswaschen nicht gebundener Serum- oder Plasmaproteine werden mit Meerrettichperoxidase (HRP) markierte, für Human-IgA spezifische Antikörper zugefügt, die mit den an β_2 GPI gebundenen Antikörpern komplexieren. Nach einem weiteren Waschschrift wird das gebundene Enzym-Antikörper-Konjugat durch Zugabe einer Lösung, die Tetramethylbenzidin (TMB) und Wasserstoffperoxid enthält, (H_2O_2) angefärbt. In den Vertiefungen entsteht eine Färbung, deren Intensität in direkter Beziehung zur Anti- β_2 GPI-Antikörper-Serum-Konzentration steht.

Die Ergebnisse erhält man durch Ablesen der optischen Dichte (OD) bzw. Extinktion in allen Vertiefungen mit einem Spektrophotometer. Es werden Kalibrationsseren mitgeliefert, deren IgA-Anti- β_2 GPI-Antikörper-Konzentrationen in A-Einheiten angegeben sind. Der Benutzer kann einen Einpunktkalibrator oder eine Vierpunkt-Kalibrierungskurve verwenden. Für die Einpunktkalibration wird die Konzentration der Kalibratorseren durch die optische Dichte (OD) des Kalibrators dividiert und ein Umrechnungsfaktor erhalten. Die OD-Werte der anderen Proben werden mit dem Umrechnungsfaktor multipliziert, um die Konzentration der IgA-Anti- β_2 GPI-Antikörper in A-Einheiten zu erhalten. Zur Mehrpunktkalibrierung wird eine lineare Regressionsanalyse mit Kalibratorwerten gegen die Kalibrator-OD-Werte durchgeführt. Die Ergebnisse für Kontrollen und Patientenproben werden mit Hilfe der Kalibrierungskurve bestimmt.

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EC/REP Authorized Representative
(Bevollmächtigter)
(Représentant autorisé)
(Representante autorizado)
(Rappresentante autorizzato)

IVD In vitro diagnostic medical device
(In-vitro-Diagnostikum)
(Dispositif de diagnostic in vitro)
(Dispositivo médico para diagnóstico in vitro)
(Dispositivo medico-diagnostico in vitro)

LOT Batch
(Charge)
(Lot)
(Lote)
(Lotto)

 Expiry date
(Verfallsdatum)
(Date de péremption)
(Fecha de caducidad)
(Data di scadenza)

 Storage conditions
(Lagerbedingungen)
(Conditions de stockage)
(Condiciones de almacenamiento)
(Condizioni di conservazione)

Xn Harmful
(Gesundheitsschädlich)
(Nocif)
(Perjudicial)
(Nocivo)

 Xi Irritant
(Reizend)
(Irritant)
(Irritante)
(Irritante)

 Biological Risk
(Biologisches Risiko)
(Risque biologique)
(Riesgo biológico)
(Rischio biologico)



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Effective: 2005-03-09

REAADS®
IgM Anti-Beta 2 Glycoprotein I Semi-Quantitative Test Kit

For *In Vitro* Diagnostic Use

An enzyme-linked immunosorbent assay (ELISA) for the semi-quantitative determination of IgM anti-Beta 2 Glycoprotein I (β_2 GPI) antibodies in human serum or citrated plasma (3.2% sodium citrate).

INTENDED USE

For the detection and semi-quantitation of IgM anti- β_2 GPI antibodies in individuals with systemic lupus erythematosus (SLE) and lupus-like disorders (anti-phospholipid syndrome).

SUMMARY AND EXPLANATION OF THE ANTI-BETA 2 GLYCOPROTEIN I TEST

Anti-phospholipid antibodies are a heterogeneous group of immunoglobulins that bind to several anionic phospholipids, including cardiolipin and phosphatidylserine.^{1,2} High serum levels of anti-phospholipid antibodies are frequently detected in patients with autoimmune (e.g., SLE) and non-autoimmune diseases, as well as in apparently healthy individuals.^{3,4} These antibodies have been associated with an increased risk for recurrent arterial and venous thrombotic events, thrombocytopenia, and fetal loss. These manifestations are the main features of the anti-phospholipid syndrome (APS).^{5,6}

Most autoimmune anti-phospholipid antibodies require a serum cofactor (β_2 GPI) for optimal binding.⁷⁻¹⁰ It has been shown that many anti-phospholipid antibodies may react to a neoepitope formed on the β_2 GPI molecule by the interaction between the phospholipid and β_2 GPI.^{11,12} Most assays for anti-phospholipid antibodies contain bovine serum as the source of cofactor. More recently, it has been shown that the binding of β_2 GPI to the microwell surface may produce a neoepitope similar to that when combined with a phospholipid and the results with this system showed a good correlation with the anti-phospholipid syndrome.¹³⁻¹⁶ The serologic detection of anti- β_2 GPI antibodies provides enhanced clinical sensitivity for thrombosis. The REAADS Anti- β_2 GPI ELISA Test Kit uses the well known ELISA format to detect anti- β_2 GPI antibodies in human serum.

Patients with positive reactions to both anti-phospholipid and anti- β_2 GPI assays were more likely to have clinical complications than those positive for only one. Higher prevalence and mean serum levels of IgM anti- β_2 GPI antibodies have been reported in autoimmune patients. In addition, anti- β_2 GPI antibodies in SLE patients correlated with clinical manifestations of anti-phospholipid syndrome.¹⁷

PRINCIPLE OF THE TEST

The test is performed as an indirect ELISA. Diluted serum or plasma samples, calibrator sera, and controls are incubated in microwells coated with purified human β_2 GPI. Incubation allows the anti- β_2 GPI antibodies present in the samples to react with the immobilized antigen. After the removal of unbound serum or plasma proteins by washing, antibodies specific for human IgM, labeled with horseradish peroxidase (HRP), are added forming complexes with the β_2 GPI bound antibodies. Following another washing step, the bound enzyme-antibody conjugate is assayed by the addition of a single solution containing tetramethylbenzidine (TMB) and hydrogen peroxide (H_2O_2) as the chromogenic substrate. Color develops in the wells at an intensity proportional to the serum concentration of anti- β_2 GPI antibodies.

Results are obtained by reading the O.D. (optical density or absorbance) of each well in a spectrophotometer. Calibrator sera are provided, with the IgM anti- β_2 GPI antibody concentrations expressed in M units. The user has the option of running either a single point calibrator or a four-point calibration curve. For single point calibration, dividing the concentration value of the calibrator sera by the O.D. value of the calibrator provides a conversion factor. The O.D. values of the other samples are multiplied by the conversion factor to obtain IgM anti- β_2 GPI antibody concentrations in M units. For multipoint calibration, perform a linear regression analysis with calibrator values against calibrator O.D.s. Controls and patient results are determined from the calibration curve.

REAGENTS

Store at 2 - 8°C. Do Not Freeze.

Each REAADS IgM Anti- β_2 GPI 96-Microwell Test Kit contains the following reagents (**volumes may vary depending on the kit size and configuration**):

- 12 x 8 stabilized β_2 GPI (from human serum) coated microwells with frame.
- 60 mL Sample Diluent IV (blue-green solution).
- 3 vials (0.250 mL) IgM β_2 GPI Calibrator Serum* (1-high, 2-moderate, 3-low) (human); see vial label for antibody concentration in M units. Calibrator 3 should be used when performing single point calibration.
- 0.250 mL IgM β_2 GPI Positive Control Serum* (human); see vial label for expected M unit range.
- 0.250 mL Normal Control Serum* (human); see vial label for expected M unit range.
- 15 mL anti-human IgM (goat) HRP-Conjugated Antibody Solution (red solution).
- 15 mL One Component Substrate Solution (TMB and H₂O₂); ready to use.
- 15 mL Stopping Solution (0.36 N sulfuric acid).
- 2 bottles (30 mL) Wash Concentrate (33X PBS/Tween).

***CAUTION: Contains sodium azide**

WARNINGS AND PRECAUTIONS

1. Human source material used to prepare the calibrators and controls included in this kit has been tested and shown to be negative to HBsAg, HCV, and HIV 1 & 2 by FDA required tests. However, all human blood derivatives, including patient samples, should be handled as potentially infectious material.
2. Do not pipette by mouth.
3. Do not smoke, eat, or drink in areas where specimens or kit reagents are handled.
4. Wear disposable gloves while handling kit reagents and wash hands thoroughly afterwards.
5. Certain components of this product contain sodium azide as a preservative. Sodium azide has been reported to form lead and copper azides when left in contact with these metals. These metal azides are explosive. Any solutions containing azide must be thoroughly flushed with copious amounts of water to prevent the build-up of explosive metal azides in the plumbing system.
6. One Component Substrate Solution can cause irritation to the eyes and skin. Use gloves when handling substrate and wash thoroughly after handling. Keep reagent away from ignition sources. Avoid contact with oxidizing agents.
7. Certain components are labeled with one or more of the following: Harmful if swallowed (R22). Avoid contact with skin and eyes (S24/25). In case of contact with eyes, rinse immediately with plenty of water and seek medical advice (S26). Wear suitable protective clothing and gloves (S36/37). Keep away from sources of ignition - no smoking (S16).

SPECIMEN COLLECTION AND PREPARATION

Serum or citrated plasma (3.2% sodium citrate) should be used as the sample matrix.

Blood should be collected by venipuncture and the serum separated from the cells by centrifugation after clot formation. If not tested immediately, the specimens should be stored at 2 to 8°C. If specimens are to be stored for more than 72 hours, they should be frozen at -20 °C or below. Avoid repeated freezing and thawing. Do not use hemolyzed, icteric, or lipemic serum or plasma as these conditions may cause aberrant results. Specimens containing visible particulate matter should be clarified by centrifugation before testing.

If citrated plasma is to be used, blood should be collected by venipuncture and the plasma separated from the cells immediately by centrifugation at 1500g for 10 minutes. The supernatant must be carefully removed after centrifugation to avoid contamination with platelets. Repeating the centrifugation and separation steps may be advisable to minimize platelet contamination. Lysed or aged platelets can lead to aberrant results. If not tested immediately, plasma samples should be stored as described for serum.

INSTRUCTIONS FOR USE

Materials Provided

REAADS IgM Anti- β_2 GPI Test Kit; see "Reagents" section for a complete list.

Materials Required but not Supplied

- Reagent grade water to prepare PBS wash solution and to zero or blank the plate reader during the final assay step
- Graduated cylinders
- Precision pipettors capable of delivering between 5 μ L and 1000 μ L, with appropriate tips
- Miscellaneous glassware appropriate for handling small volumes
- Flasks or bottles, 1 liter
- Wash bottles, preferably with the tip partially cut back to provide a wide stream, or an automated or semi-automated washing system
- Disposable gloves
- Plate-reading spectrophotometer capable of reading absorbance at 450 nm (650 nm reference if dual beam)
- Multichannel pipettors capable of delivering to 8 wells simultaneously
- Microdilution tubes and a 96-well microdilution tube holder for sample dilutions and rapid delivery to microwell plate

Procedural Notes

1. Bring serum or plasma samples and kit reagents to room temperature and mix well before using; avoid foaming. Return all unused samples and reagents to refrigerated storage as soon as possible.
2. All dilutions of calibrators, controls, and test sera or plasma must be made just prior to use in the assay.
3. A single water blank well can be included in each plate with each run. No sample or kit reagents are to be added to this well. Instead, add 200 μ L of reagent grade water to the well immediately prior to reading the plate in the spectrophotometer. The plate reader should be programmed to "zero" or "blank" against an air or a water well.
4. Good washing technique is critical for optimal performance of the assay. Adequate washing is best accomplished by directing a forceful stream of wash solution into the bottom of the microwells from a plastic squeeze bottle with a wide tip. Wash solution in the water blank well will not interfere with the procedure. An automated plate washing system can also be used.
5. IMPORTANT: Failure to adequately remove residual wash solution can cause inconsistent color development of the Substrate Solution.
6. Use a multichannel pipettor capable of delivering to 8 wells simultaneously when possible. This speeds the process and provides more uniform incubation and reaction times for all wells.
7. Carefully controlled timing of all steps is critical. All calibrators, controls, and samples must be added within a five minute period. Batch size of samples should not be larger than the amount that can be added within this time period.
8. For all incubations, the start of the incubation period begins with the completion of reagent or sample addition.
9. Addition of all samples and reagents should be performed at the same rate and in the same sequence.
10. Incubation temperatures above or below normal room temperature (18 to 26 °C) may contribute to inaccurate results.
11. Avoid microbial and cross-contamination of reagents when opening and removing aliquots from the primary vials.
12. Do not use kit components beyond expiration date.
13. Do not use kit components from different kit lot numbers.

Reagent Preparation

Wash Solution (PBS/Tween): Measure 30 mL of Wash Concentrate and dilute to 1 liter with reagent grade water. The pH of the final solution should be 7.35 ± 0.1 . Store unused wash solution in the refrigerator at 2 - 8 °C. Discard if the solution shows signs of microbial or cross-contamination.

Assay Procedure

1. The assay can be performed with a single point calibration (Calibrator 3) or a four-point calibration curve (Calibrators 1, 2, and 3 plus sample diluent/reagent blank as Calibrator 4 equal to 0 M units). A reagent blank control should also be run with the single point and multipoint calibration method. Sample Diluent without serum or plasma is added to the well. This well will be treated the same as a control or patient sample in subsequent assay steps.
2. Remove any microwell strips that will not be used from the frame and store them in the bag provided.
3. Prepare a 1:50 dilution of the calibrators, controls, and patient samples in sample diluent (blue-green solution); e.g., 10 μ L sample added to 490 μ L Sample Diluent equals a 1:50 sample dilution.

4. Add 100 μ L of diluted calibrators (including the reagent blank/Calibrator 4), controls, and patient samples to the appropriate microwells.
5. Incubate 15 minutes at room temperature. After the incubation is complete, carefully invert the microwells and empty the sample fluid. Do not allow samples to contaminate other microwells.
6. Wash 4 times with wash solution. Each well should be filled with wash solution per wash. Invert microwells between each wash to empty fluid. Use a snapping motion of the wrist to shake the liquid from the wells. To retain microwell modules during washing, the frame must be squeezed at the top and bottom of the longest sides. Blot on absorbent paper to remove residual wash fluid. Do not allow wells to dry out between steps.
7. Add 100 μ L anti-human IgM HRP-Conjugated Antibody Solution (red) to the wells.
8. Incubate for 15 minutes at room temperature. After the incubation is complete, carefully invert the microwells and empty the conjugate solution.
9. Wash 4 times with wash solution, as in step 6. Use a snapping motion to drain the liquid and blot on absorbent towels after the final wash. Do not allow the wells to dry out.
10. Add 100 μ L One Component Substrate to each well and incubate for 10 minutes at room temperature. Add substrate to the wells at a steady rate. Blue color will develop in wells with positive samples.
11. Add 100 μ L Stopping Solution (0.36 N sulfuric acid) to each well to stop the enzyme reaction. Be sure to add the acid to the wells in the same order and at the same rate as the Substrate was added. Blue Substrate will turn yellow and colorless solution will remain colorless. Blank or zero the plate reader against an air or a water blank well. Read the O.D. of each well at 450 nm (and 650 nm reference if dual beam). The O.D. values should be measured within 5 minutes of the addition of the Stopping Solution.

RESULTS

Single Point Calibration

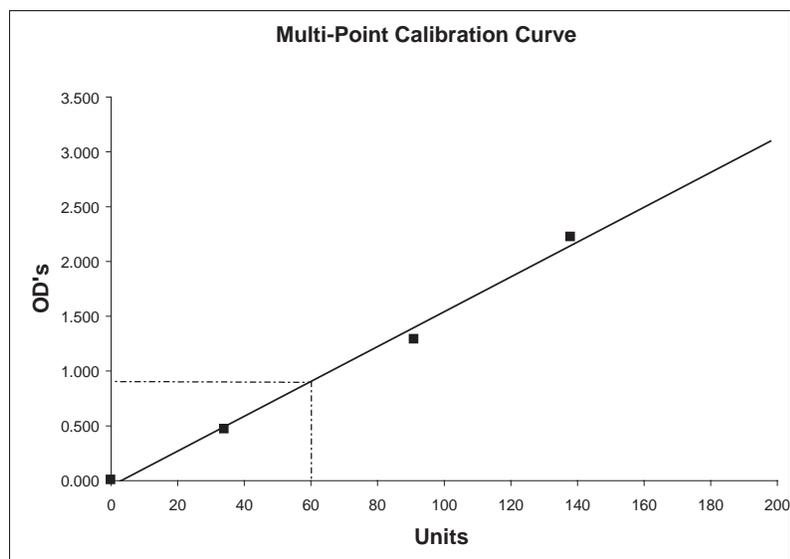
1. Calculate the mean O.D. values if duplicates of Calibrator 3, Controls and patient samples were performed.
2. Divide the concentration value of Calibrator 3 (printed on vial label) by the O.D. or mean O.D. value of the calibrator serum to obtain the conversion factor.
3. Multiply the O.D. or mean O.D. values for each of the controls and patient samples by the conversion factor to obtain an anti- β_2 GPI antibody concentration value expressed in M units.

$\text{Conversion Factor} = \frac{\text{Anti-}\beta_2\text{GPI Concentration of Calibrator 3}}{\text{Absorbance Value of the Calibrator 3 (O.D.)}}$ $\text{Anti-}\beta_2\text{GPI Concentration of Sample} = \text{Conversion Factor} \times \text{Absorbance of the Sample (O.D.)}$
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4. The Conversion Factor must be calculated for the Calibrator 3 for each assay run. Using a Conversion Factor from another assay will invalidate the results.

Multi-Point Curve Calibration

1. Calculate the mean O.D. values if duplicates of the calibrators, controls and patient samples were performed.
2. Perform linear regression analysis with the four calibrator values (See vial labels for M units. Calibrator 4 [sample diluent] is equal to 0 M units) against the mean O.D.s for each calibrator.
3. The calibrator curve can be plotted either automatically using a validated software program or manually with graph paper. It is recommended to use a zero intercept when generating the regression line to avoid negative values. If this option is not available, any negative values should be reported as zero units. When generating the curve manually, draw a best fit line through the plotted points using a zero intercept.
4. Determine the control and patient sample values from the calibrator curve.
5. Example of a multi-point curve calibration.



Using the example calibration curve provided, a specimen O.D. of 0.860 at 450nm would correspond to a calculated value of 60 units. The calibration curve provided is an example only and should not be used to calculate patient results. A new calibration curve should be performed with every test run.

Quality Control

1. The O.D. value of the Calibrator 3 should be at least 0.400 to assure that the kit is functioning properly. Calibrator 3 O.D. readings of less than 0.400 may indicate that the kit is no longer suitable for use.
2. The O.D. of Calibrator 4 or reagent blank should be less than 0.050 when the spectrophotometer has been blanked against the water well. Readings greater than 0.050 may indicate possible reagent contamination or inadequate plate washing.
3. The anti- β_2 GPI values obtained for the control sera should be within the ranges indicated on the vial labels. Occasional small deviations outside these ranges are acceptable.
4. O.D. values for duplicates of the controls or patient samples should be within 20% of the mean O.D. value for samples with absorbance readings greater than 0.200.
5. Each laboratory should periodically determine its own normal cut-off values for the appropriate population of patients.
6. Samples with anti- β_2 GPI values greater than 200 M units may be reported as "greater than 200 M units."
7. Assure that all quality control parameters have been met before reporting test results.

NORMAL RANGE

Serum samples from 120 healthy blood donors were tested for IgM anti- β_2 GPI antibodies. The following normal range was established:

- Less than 20 M units

PERFORMANCE CHARACTERISTICS

Clinical Specificity

Normal Samples:

Serum samples from 120 healthy blood donors were assayed for the presence of IgM anti- β_2 GPI antibodies. Using the established cutoff value of 20 M units, this normal population demonstrated 93% specificity (mean value = 7.7 M units).

Serum samples from 41 infectious disease (syphilis), 42 progressive systemic sclerosis (PSS), and 42 rheumatoid arthritis (RA) patients were assayed for the presence of IgM anti- β_2 GPI antibodies. These patient groups demonstrated similar results compared to the healthy blood donor population (mean values = 9.7, 10.1, and 11.1 M units respectively). Results of these groups along with the healthy blood donors are summarized in the table below.

IgM anti- β_2 GPI	Healthy	Infectious (syphilis)	PSS	RA
# of Samples (n)	120	41	42	42
Mean (M units)	7.7	9.7	10.1	11.1
Standard Deviation	9.5	17.9	15.1	16
% Negative	92.5%	95.0%	92.9%	88.1%

Clinical Sensitivity

Systemic Lupus Erythematosus (SLE):

Serum samples from 40 unselected (consecutive) patients with SLE were tested with this kit. Eight of the samples (sensitivity of 20%) were positive for IgM anti- β_2 GPI antibodies (mean value = 14 M units).

Serum samples from 12 selected female patients with SLE who had a clinical history of thrombosis, thrombocytopenia or recurrent fetal loss were evaluated for IgM anti- β_2 GPI antibodies. Five of the samples (sensitivity of 42%) were positive in this population (mean value = 24 M units).

SLE Controls:

Serum samples from five selected female patients with SLE who had history of thrombocytopenia (no thrombosis) were tested for IgM anti- β_2 GPI antibodies. None tested positive, and the resulting mean value was 6 M units for this group.

Serum samples from nine selected female patients with SLE who were known not to have had thrombotic episodes, nor any other feature of the anti-phospholipid syndrome, were tested in the assay. One sample (11%) was weak positive for IgM anti- β_2 GPI antibodies, with a mean value of 9 M units for this group.

Primary Anti-phospholipid Syndrome (APS):

Serum samples from nine patients with the diagnosis of primary anti-phospholipid syndrome (APS) were tested on the REAADS IgM anti- β_2 GPI assay (Lot GW-001). Three of the nine samples resulted positive (33.3% sensitivity) with a mean value of 32.7 M units. A summary of sensitivity testing is presented below:

	Unselected SLE	Selected SLE		Primary APS
		No Thrombosis	Thrombosis	
# of Samples (n)	40	14	12	9
Mean (M units)	13.9	8.0	24.4	32.7
Standard Deviation	18.1	10.3	24.8	37.3
% Positive	20.0%	7.1%	41.7%	33.3%

Technical Performance Comparison

Two disease populations (unselected SLE and primary APS) were tested on REAADS IgM anti- β_2 GPI ELISA and a predicate device to study the correlation between positive and negative results. The results are summarized in the table presented below.

REAADS IgM anti- β_2 GPI

		Negative	Positive		
Predicate Device IgM anti-β_2GPI	Negative	35	4	Relative Sensitivity	70%
	Positive	3	7	Relative Specificity	90%
				Agreement:	86%

Precision

Three samples with known M unit values (one low, one moderate, and one high) were assayed in 23 replicates on three different occasions. The mean intra-assay and inter-assay coefficients of variation (%CVs) are presented in the table below. The reported intra-assay coefficient of variation is the mean of the three separate intra-assay CVs. Inter-assay CV is the coefficient of variation obtained from three plates from one lot.

Value Range	Mean Intra-assay %CV	Mean Inter-assay %CV
Low (13 - 16 M units)	3.8%	6.1%
Moderate (44 - 56 M units)	3.5%	4.5%
High (73 - 82 M units)	2.4%	3.4%

LIMITATIONS OF THE TEST

The anti- β_2 GPI antibody concentration values obtained from this assay are an aid to diagnosis only. Each physician must interpret these results in light of the patient's history, physical findings, and other diagnostic procedures. If clinical findings suggest the presence of anti-phospholipid antibodies and the patient is negative for anti- β_2 GPI antibodies, some investigators recommend testing for anti-cardiolipin antibodies, anti-phosphatidylserine antibodies, and the lupus anticoagulant to confirm the negative result. A patient may be considered positive for anti-phospholipid antibodies if one or all of the tests give positive results.

Warranty

This product is warranted to perform as described in this package insert. Corgenix, Inc. disclaims any implied warranty of merchantability or fitness for a particular use, and in no event shall Corgenix, Inc. be liable for consequential damage.

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DEUTSCH

REAADS®

IgM Anti-Beta 2 Glycoprotein I Semi-Quantitative Test Kit

In-vitro-Diagnostikum

Ein enzymimmunologischer Test (ELISA) zur semiquantitativen Bestimmung von IgM-Anti-Beta 2 Glykoprotein I (β_2 GPI)-Antikörpern in Humanserum oder Zitratplasma (3,2 % Natriumzitat).

ANWENDUNGSBEREICH

Nachweis und semiquantitative Bestimmung von IgM-Anti- β_2 GPI-Antikörpern bei Personen mit Lupus erythematosus (SLE) oder lupusartigen Erkrankungen (z.B. Antiphospholipid-Syndrom).

TESTPRINZIP

Der Test wird als indirekter ELISA durchgeführt. Proben verdünnten Serums oder Plasmas, Kalibratorseren und Kontrollen werden in Mikrovertiefungen inkubiert, die mit gereinigtem Human β_2 GPI beschichtet sind. Die Inkubation ermöglicht eine Reaktion der in den Proben enthaltenen Anti- β_2 GPI-Antikörper mit dem immobilisierten Antigen. Nach dem Auswaschen nicht gebundener Serum- oder Plasmaproteine werden mit Meerrettichperoxidase (HRP) markierte, für Human-IgM spezifische Antikörper zugefügt, die mit den an β_2 GPI gebundenen Antikörpern komplexieren. Nach einem weiteren Waschschrift wird das gebundene Enzym-Antikörper-Konjugat durch Zugabe einer Lösung, die Tetramethylbenzidin (TMB) und Wasserstoffperoxid enthält, (H_2O_2) angefärbt. In den Vertiefungen entsteht eine Färbung, deren Intensität in direkter Beziehung zur Anti- β_2 GPI-Antikörper-Serum-Konzentration steht.

Die Ergebnisse erhält man durch Ablesen der optischen Dichte (OD) bzw. Extinktion in allen Vertiefungen mit einem Spektrophotometer. Es werden Kalibrationsseren mit geliefert, deren IgM-Anti- β_2 GPI-Antikörper-Konzentrationen in M-Einheiten angegeben sind. Der Benutzer kann einen Einpunktkalibrator oder eine Vierpunkt-Kalibrierungskurve verwenden. Für die Einpunktkalibration wird die Konzentration der Kalibratorseren durch die optische Dichte (OD) des Kalibrators dividiert und ein Umrechnungsfaktor erhalten. Die OD-Werte der anderen Proben werden mit dem Umrechnungsfaktor multipliziert, um die Konzentration der IgM-Anti- β_2 GPI-Antikörper in M-Einheiten zu erhalten. Zur Mehrpunktkalibration wird eine lineare Regressionsanalyse mit Kalibratorwerten gegen die Kalibrator-OD-Werte durchgeführt. Die Ergebnisse für Kontrollen und Patientenproben werden mit Hilfe der Kalibrierungskurve bestimmt.

REAGENZIEN

Bei 2-8 °C aufbewahren. Nicht einfrieren!

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IVD In vitro diagnostic medical device
(In-vitro-Diagnostikum)
(Dispositif de diagnostic in vitro)
(Dispositivo médico para diagnóstico in vitro)
(Dispositivo medico-diagnostico in vitro)

LOT Batch
(Charge)
(Lot)
(Lote)
(Lotto)

 Expiry date
(Verfallsdatum)
(Date de péremption)
(Fecha de caducidad)
(Data di scadenza)

 Storage conditions
(Lagerbedingungen)
(Conditions de stockage)
(Condiciones de almacenamiento)
(Condizioni di conservazione)

Xn Harmful
(Gesundheitsschädlich)
(Nocif)
(Perjudicial)
(Nocivo)

Xi Irritant
(Reizend)
(Irritant)
(Irritante)
(Irritante)

 Biological Risk
(Biologisches Risiko)
(Risque biologique)
(Riesgo biológico)
(Rischio biologico)



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