Affinity Biologicals™ INC.

REPRESENTATIVE DATASHEET

VisuLize™ FVIII Antigen Kit

96 Test Enzyme Immunoassay Kit for
Factor VIII (FVIII) antigen.

For *In Vitro* Diagnostic Use.

Product # FVIII-AG CE

Store at 2–8°C. Do not freeze.

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INTENDED USE

The *VisuLize*™ FVIII Antigen kit is an Enzyme Immunoassay for the quantitative determination of Factor VIII antigen in human plasma samples and Factor VIII concentrates using the double antibody enzyme linked immuno-sorbent assay (ELISA).

SUMMARY

Factor VIII (formerly referred to as antihaemophilic globulin and Factor VIII:C) is a large glycoprotein with a molecular weight of 320000 daltons that circulates in plasma at a concentration of approximately 200 ng/mL.[1,2] FVIII is stabilized by association with von Willebrand Factor to form a FVIII-vWF complex required for the normal survival of FVIII in vivo ($t_{1/2}$ of 8-12 hours).[3] FVIII is a pro-cofactor that is activated through limited proteolysis by thrombin. In this process FVIIIa dissociates from vWF to combine with activated Factor IX, calcium and a phospholipid surface where it is an essential cofactor in the assembly of the Factor X activator complex.[4,5] Once dissociated from vWF, FVIIIa is susceptible to inactivation by activated Protein C and by non-enzymatic decay.[4]

The biological importance of Factor VIII is demonstrated in Hemophilia A, a congenital bleeding disorder occurring primarily in males that results from an X-chromosome-linked deficiency of FVIII.[6] The prevalence of Hemophilia A has been estimated to be between 1/5000 and 1/10000.[6] The severity of the deficiency generally correlates with the severity of the disease. Individuals with <1% Factor VIII activity are classified as severe patients, those with between 1 and 5% Factor VIII activity are classified as moderate and those with between 5 and 40% Factor VIII activity are classified as mild hemophiliacs.[7] Some Hemophiliacs produce a FVIII protein that is partially or totally inactive. In these cases, the Factor VIII activity is low but the antigen levels are normal or near normal. These patients, comprising approximately 5% of Hemophilia A patients, are termed cross-reacting material (CRM)-positive.[8] The production of neutralizing antibodies to FVIII also occurs in 5-20% of Hemophiliacs.[9,10]

The laboratory diagnosis of Factor VIII deficiency typically involves quantitative determinations based on procoagulant levels (functional activity of Factor VIII typically measured by clotting assay). Clinical bleeding symptoms may also be used in the diagnosis and classification, however, the quantitative procoagulant measurement is the preferred method of classifying the severity of hemophilia.[7] An ELISA for Factor VIII antigen may be used in conjunction with the functional assays in the assessment of Factor VIII replacement therapies, assessment of carrier status as well as to distinguish those patients that may be deemed CRM-Positive.

PRINCIPLE OF ENZYME IMMUNOASSAY

Strip wells are pre-coated with sheep polyclonal antibody to human FVIII. Plasma samples are diluted and applied to the wells. The FVIII antigen present binds to the coated antibody. After washing away unbound material, peroxidase-labeled sheep detecting antibody is applied and allowed to bind to the captured FVIII. The wells are again washed and a solution of TMB (the peroxidase substrate tetramethylbenzidine) is applied and allowed to react for a fixed period of time. A blue color develops which changes to yellow upon quenching the reaction with acid. The color formed is measured spectrophotometrically in a microplate reader at 450 nm. The absorbance at 450 nm is directly proportional to the quantity of FVIII antigen captured onto the well. The assay is calibrated using the calibrator plasma provided in the kit.

REAGENTS

A. Description of Reagent Items

Item 1: Foil pouch containing 6 strips, each containing 16 wells coated with sheep antibody to human FVIII.
Item 2: 2 vials of Calibrator Plasma, each lyophilized from 1 mL plasma.
Item 3: 2 vials of Control Plasma A, each lyophilized from 1 mL plasma.
Item 4: 2 vials of Control Plasma B, each lyophilized from 1 mL plasma.
Item 5: 1 vial containing 50 mL of 20X Wash Buffer Concentrate.
Item 6: 3 vials, each containing 20 mL of buffered Sample Diluent.
Item 7: 1 vial containing 12 mL peroxidase-labeled sheep detecting antibody.
Item 8: 1 vial containing 12 mL of tetramethylbenzidine (TMB) substrate.
Item 9: 1 vial containing 12 mL Stop Solution (0.2 M Sulphuric acid).

B. Caution and Warning

This kit is intended for use by personnel trained in laboratory procedures and universal precautions for the use of chemicals and potentially biohazardous substances. Some items contain human source material. Each unit of source plasma used in the preparation of this product has been tested by FDA approved methods and found negative for the presence of Human Immunodeficiency Virus (HIV) Type I and Type II, Hepatitis B surface antigen (HB_SAg) as well as for Hepatitis C (HCV). However, no test can offer complete assurance that products derived from human blood will not transmit infectious diseases. As with all materials of human origin, this product should be handled as a potentially infectious material.

The substrate TMB (tetramethylbenzidine) has reduced toxicity, but precautions should still be taken to avoid direct contact. The use of gloves and safety glasses is recommended.

The Stop Solution contains dilute sulphuric acid (0.2 M), which is corrosive. The use of gloves and safety glasses is recommended.

The disposal of waste materials must be carried out according to current local regulations.

For a Material Safety Data Sheet for this product contact Affinity Biologicals Inc.

C. Reagent Preparation

Item 1 (Antibody-coated strips with frame): Just prior to use, open pouch and remove strips and frame. Unused strips should be replaced in the pouch and resealed. Strips may be used directly, see Procedure section C: Assay Procedure.

Item 2 (Calibrator plasma): Reconstitute one vial with 1.0 mL of reagent grade water. Allow contents to dissolve for 15 minutes at room temperature with occasional swirling. Stability after reconstitution is 4 hours at ambient (18-25°C), or 30 days at –20° C.

Items 3 and 4 (Control plasmas): Reconstitute one vial of each plasma with 1.0 mL of reagent grade water. Allow contents to dissolve for 15 minutes at room temperature with occasional swirling. Stability after reconstitution is 4 hours at ambient (18-25°C), or 30 days at –20° C.

Item 5 (20X Wash Buffer Concentrate): Allow vial to warm to room temperature before use. Ensure any crystals that may have formed are dissolved before proceeding. If necessary the vial can be warmed to 37°C until all crystals have dissolved. Dilute the concentrate 1/20 before use. For every 2 strips (32 wells), add 16 mL concentrate to 304 mL reagent grade water and mix. Stability after dilution is 1 week at 2–8°C.

Items 6-9 are supplied ready to use.

D. Storage and Stability

Intact kits and un-reconstituted reagents are stable until the expiration date stated on the box and individual reagent labels when stored at 2–8°C.

SPECIMEN COLLECTION

Blood is collected into 3.2% Buffered Citrate anticoagulant tubes at a ratio of 9 volumes blood to 1 volume anticoagulant and gently mixed by inversion. Centrifuge at a minimum of 1500 x g for 15 minutes (NCCLS Guideline H21-A4[11]). Remove supernatant plasma and use within 4 hours or freeze below –20°C for up to 1 month.

PROCEDURE

A. Material Provided

Foil pouch containing 6 strips of antibody coated wells.
Calibrator Plasma, lyophilized.
Control Plasma A, lyophilized.
Control Plasma B, lyophilized.
20X Wash Buffer Concentrate.
Sample Diluent.
Detecting antibody solution.
TMB substrate.
Stop Solution.
Adhesive Plate Sealer.

B. Additional Material Required (but not provided)

Reagent grade water for reconstitution and for dilution
Single-channel adjustable volume pipettes
Multi-channel pipettes
Pipette Tips
Laboratory timer
Microplate strip-well washer device
Microplate compatible spectrophotometer capable of 450 nm.

C. Assay Procedure

PROCEDURAL NOTES:

- Reconstitute reagents as described in REAGENTS, Section C, Reagent Preparation. Allow reagents to warm to room temperature before use.
- It is recommended that all calibrator, control and test sample dilutions be run in duplicate and that each run include a buffer blank (see Assay Calibration section).
- All dilutions must be made just prior to use in the assay.
- Do not allow the wells to become dry at any time. Keep plate covered during incubations.
- Plasma samples should not be applied at dilutions lower than 1/4.
- Do not use kit components from different lot numbers.
- Incubation temperatures above or below normal room temperature (18 -25°C) may contribute to inaccurate results.
- Do not use kit components beyond expiration date
- Used strips must be discarded and not re-used.

1. **Preparation of Calibrator Plasma Dilutions:** Dilute the Calibrator Plasma (reconstituted Item 2) into Sample diluent (Item 6) as detailed in Table 1 below: (NOTE: 100% = 1.0 IU/mL)

TABLE 1:

Dilution	Calibrator Plasma	Sample Diluent
100%	175 µL	525 µL
50%	350 µL of 100%	350 µL
25%	350 µL of 50%	350 µL
12.5%	350 µL of 25%	350 µL
6.25%	350 µL of 12.5%	350 µL
3.13%	350 µL of 6.25%	350 µL
1.56%	350 µL of 3.13%	350 µL
0.79%	350 µL of 1.56%	350 µL

2. **Control plasma A** (reconstituted Item 3) and **normal test plasmas** are diluted 1/8 and 1/16. Add 100 µL plasma into 700 µL sample diluent (Item 6), mix, then add 350 µL of this 1/8 dilution into 350 µL sample diluent to obtain the 1/16 dilution. **Control Plasma B** (reconstituted Item 4) and samples low in FVIII antigen (Haemophiliac samples) should be run at lower dilutions of 1/4 and 1/8. Add 175 µL plasma into 525 µL sample diluent (Item 6), mix, then add 350 µL of this 1/4 dilution into 350 µL sample diluent to obtain the 1/8 dilution.

3. **Assay**

PLATE PREPARATION	Place desired number of strips into frame.	
STEP	Pipette into each pre-coated well:	
FVIII CAPTURE	Test Sample (run in duplicate)	100 µL
	Cover strips with the plate sealer and incubate 1 hour at ambient temperature.	
Empty wells and wash with 300 µl diluted wash buffer 3 times.		
DETECTING ANTIBODY	Detecting Antibody Solution (Item 7)	100 µL
	Cover strips with the plate sealer and incubate 45 minutes at ambient temperature.	
Empty wells and wash with 300 µl diluted wash buffer 3 times.		
COLOR DEVELOPMENT	TMB Substrate (Item 8)	100 µL
	Allow color to develop for **exactly** 10 minutes at ambient temperature.	
	Stop Solution (Item 9)	100 µL (Add to each well in same order in which the TMB was added)
Read plate at a wavelength of 450 nm within 30 minutes of adding Stop Solution. If necessary, keep plate frame for use with any unused strips. Discard used strips.		

CALIBRATION

A. Assay Calibration

The FVIII antigen value stated on the Calibrator Plasma vial has been determined by comparison to a secondary standard that is traceable to the WHO International standard for FVIII antigen 02/150. This antigen value should be used as the concentration of the initial dilution of the calibrator plasma.
It is recommended that the plate be blanked on wells that have received Sample Diluent alone instead of diluted sample (reagent blank wells).

B. Reference Curve and Calculation of Results

The reference curve is a log-log plot of the mean absorbance values (y axis) versus the FVIII antigen concentration (x axis). The Factor VIII antigen content of test samples and controls can be read from the reference curve and multiplied by the appropriate dilution factor. Under the conditions described here, a sample diluted 1/4 will have a dilution factor of 1, a dilution of 1/8 will have a dilution factor of 2, and a dilution of 1/16 has a dilution factor of 4.

Example: Test plasma when diluted 1/8 gives an absorbance corresponding to 45% when read from the reference curve. This value would be multiplied by a dilution factor of 2 to obtain the corrected value of 90%.

QUALITY CONTROL

The supplied Control Plasmas (Item 3 and 4) should be assayed with every series of samples that are run. The FVIII antigen values obtained for test samples should be considered suspect if the values obtained for the control plasmas fall outside of the range stated on the Control Plasma labels.

LIMITATIONS AND INTERFERENCES

The Factor VIII antigen values obtained using this assay should not be used in isolation to diagnose disease. Patient history, clinical presentation and findings from other diagnostic procedures should also be considered. Clinically significant states are known to exist in which plasma Factor VIII antigen levels are normal or near-normal in the presence of a significant reduction in Factor VIII activity.[8]
This kit has been developed for use with citrated plasma. The use of samples containing anticoagulants other than 3.2% sodium citrate is not recommended.
Assay interference due to the presence of drugs or due to the presence of heterophilic antibodies such as Lupus Anticoagulant (LA) has not been reported, however, the potential for interference by high levels of heterophilic antibodies cannot be excluded. The presence of Rheumatoid Factor in test samples will cause interference in the assay. The theoretical possibility of test samples containing antibodies to sheep immunoglobulin may also interfere in the assay.

EXPECTED VALUES

The normal range for Factor VIII as reported in the literature is 0.5-1.8 IU/mL.[12] Each laboratory should determine a normal range independently but results from three lots measured in 99 healthy individuals indicate a normal reference interval for FVIII antigen of 0.64-1.89 IU/mL (mean 1.268 IU/mL, SD = 0.3116).

PERFORMANCE CHARACTERISTICS

A. Specificity

This assay measures Factor VIII antigen in human plasma, therapeutic Factor VIII concentrates and recombinant Factor VIII preparations.

B. Detection Limit

When assay is performed as indicated in Section C, Assay Procedure, the detection limit of this assay is 0.008 IU/mL (0.8 %) Factor VIII antigen. The upper limit of detection may vary with each lot of kit depending on the assayed value of the calibrator plasma supplied in the kit. Samples with values outside the range of the reference curve should be re-tested at an appropriate dilution to obtain accurate results.

C. Method Comparison:

The average results of three lots of the *VisuLize*™ Factor VIII Antigen kit were compared internally to the Coamatic® FVIII Assay on 142 patient samples containing Factor VIII levels ranging across the entire detection range. The correlation co-efficient (r) was 0.970 (R^2 =0.940, y=1.2059x + 0.1053). The *VisuLize*™ Factor VIII Antigen kit was also compared at two external testing sites to the Coamatic® FVIII Assay on patient samples with Factor VIII levels ranging across the entire detection range. At external site #1, 110 samples were tested and the correlation co-efficient (r) was 0.969 (R^2 =0.9381 y=1.2261x + 0.1085). At external site #2, 81 samples were tested and the correlation co-efficient (r) was 0.974 (R^2 =0.9488, y=1.1768x + 0.0242).

D. Precision

Intra-assay Precision, Method 1: In each of three lots of product, normal and abnormal plasma samples were tested in 4 assays total with 38 replicates per sample per plate. The mean coefficient of variation (CV) from all results was 4.56%

Intra-assay Precision, Method 2: Three plasmas with different Factor VIII antigen concentrations were tested in replicates of 8 in 20 assay events using 3 lots of product. The intra-assay coefficient of variation (CV) was calculated according to NCCLS Guideline EP5-A[13] and is indicated in the summary below for each Factor VIII level. The mean CV from all results by this method was 4.51%.

	Lot 1	Lot 2	Lot 3
1.35-1.75 IU/mL sample	2.17%	2.85%	3.89%
0.6-0.9 IU/mL sample	2.85%	3.33%	5.19%
<0.055 IU/mL sample	5.23%	6.83%	8.25%

Inter-assay Precision: Three plasmas with different Factor VIII antigen concentrations were tested in replicates of 8 in 20 assay events using 3 lots of product. The inter-assay coefficient of variation (CV) was calculated according to NCCLS Guideline EP5-A[13] and is indicated in the summary below for each Factor VIII level. The mean CV from all results by this method was 4.69%

	Lot 1	Lot 2	Lot 3
1.35-1.75 IU/mL sample	2.12%	3.92%	4.11%
0.6-0.9 IU/mL sample	1.63%	3.19%	7.30%
<0.055 IU/mL sample	6.79%	5.49%	7.71%

E. Lot-to-Lot Variability

94 control samples with Factor VIII antigen values ranging from 0.23-2.3 IU/mL were tested in duplicate on three lots to determine assay precision between lots. The mean lot-to-lot variability was 7.78%.

SYMBOL LEGEND [14]

IVD For *in vitro* diagnostic use

LOT Batch code

EC REP Authorized Representative


Expiry date

REF Catalogue Number


Temperature limitation


Manufacturer


Consult instructions for use


Contains sufficient for <n> tests

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Limited Warranty: This product is warranted to perform in accordance with its labelling and literature. Affinity Biologicals Inc. disclaims any implied warranty of merchantability or fitness for any other purposes, and in no event will Affinity Biologicals Inc. be liable for any consequential damages arising out of aforesaid express warranty.

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