CHROMOGENIX

COAMATIC® Heparin - 82 3393 63

ENGLISH - Insert revision 12/2008

CE

Intended use

For the quantitative determination of unfractionated heparin (UF Heparin) or low molecular weight heparin (LMW Heparin) in human citrated plasma using automated and microplate methods.

Background and summary

Heparin is the most frequently used antithrombotic therapeutic.
The biological activity of this sulfated glycosaminoglycan resides in its ability to accelerate (up to 2000-fold) the inhibitory effect of antithrombin (AT) on the coagulation proteases.
The amount of LMW Heparin or UF Heparin is determined from the anti-FXa activity expressed by the [AT ● Heparin] complex formed in plasma.[1-3]

Measuring principle

Heparin + AT ⟶ [AT ● Heparin]

[AT ● Heparin] + FXa (excess) ⟶ [FXa ● AT ● Heparin] + FXa (residual)

S-2732 ⟶ Peptide + pNA

Factor Xa (FXa) is added to a mixture of undiluted plasma and the chromogenic substrate S-2732.
When Heparin and AT are complexed, two competing reactions occur simultaneously:
1. Inhibition of FXa by the [AT ● Heparin] complex.
2. Reaction of FXa with S-2732 resulting in cleavage of pNA.
   The pNA release measured at 405 nm is inversely proportional to the heparin level in the sample.[1]

In order to reduce the influence from heparin antagonists, such as platelet factor 4 (PF4), dextran sulfate is included in the reaction mixture.[4]

Reagents

1. **S-2732, 15 mg** — 2 vials
   Chromogenic substrate, Suc-Ile-Glu(γ-pip)-Gly-Arg-pNA ● HCl lyophilized with detergent and mannitol as bulking agent.
2. **Factor Xa, 35 nkat** — 2 vials
   Lyophilized bovine FXa containing Tris buffer, EDTA, NaCl, dextran sulfate and bovine serum albumin.

PRECAUTIONS AND WARNINGS:
Avoid contact with skin and eyes (S24/25).
Do not empty into drains (S29).
Wear suitable protective clothing (S36).
This product is for ***in vitro*** diagnostic use.

Reagent preparation:
For the microplate method reconstitute REAGENTS 1 and 2 with 5.0 mL of water (see REAGENTS 3). Replace the stoppers and swirl gently. Make sure of the complete reconstitution of the product. Keep reagent at 15-25°C for 10-30 min and invert before use.

NOTE: Other reagent reconstitution volumes may apply for automated methods. (See section: INSTRUMENT APPLICATIONS). The reagents are not interchangeable between lots.

Reagents required but not provided:
3. Deionized water filtered through 0.22 mm or NCCLS type II water.[5]
4. Acetic acid 20% or citric acid 2% (end-point method).
5. Saline (0.9% NaCl).
6. Human normal plasma.
7. Calibrator plasma for LMW Heparin and/or UF Heparin calibrated against International Standards.
8. Controls for LMW Heparin and/or UF Heparin activity.

NOTE: Antithrombin reagent and tris buffer is required for the ACL Hundred/Thousand Series method (the assay is run as a two stage method with the addition of antithrombin). See the instrument Application Sheet for specific information.

Materials required but not provided:
— Spectrophotometer 405 nm (and 490 nm for the microplate procedure)
— Incubator 37°C
— Microplates*
— Centrifuge, 2000 x g
— Plastic test tubes
— Stopwatch
— Vortex mixer
— Calibrated pipettes
— Linear graph paper

*NOTE: Do not use microplates intended for coating

Storage conditions and stability

The sealed reagents are stable at 2-8°C until the expiry date printed on the label.
1. **S-2732**
   Stability after reconstitution: 3 months at +2-8°C in the original vial.
2. **Factor Xa**
   Stability after reconstitution: 3 months at +2-8°C in the original vial.

WARNING: Do not use reagents beyond the expiry date printed on the package label. Substrate - Avoid exposure to light. Discard the substrate solution if it appears yellow. Avoid contamination by microorganisms.

Specimen collection

Nine parts of freshly drawn venous blood is collected into one part trisodium citrate. Centrifugation: 2000 x g for 20 minutes at 20-25°C. Refer to NCCLS document H21-A2 for further instructions on specimen collection, handling and storage.[6]

Quality controls

Two levels of heparin controls, calibrated against International standards, are recommended for a complete quality control program.[7] Each laboratory should establish its own mean and standard deviation and should establish a quality program to monitor laboratory testing. Controls should be analyzed at least once every 8 hour shift in accordance with good laboratory practice. Refer in Westgard ***et al*** for identification and resolution for out-of-control situations.[8]

Results

Heparin results are reported in activity (IU/mL).

Expected values

To obtain an optimal effect with minimum risk of bleeding or thromboembolic complications the heparin should be in the range recommended by the manufacturer.[9]

Procedures

All conditions included in this package insert are referred to Microplate method and Cobas Mira. Detailed instrument settings including instructions for preparation of the reagents for a variety of automated instruments are available on request from Chromogenix

Assay condition for microplate and test tube techniques

Dilutions of samples and controls.

Samples/controls/standards	100 µL
Water (see REAGENTS 3)	300 µL
Mix well	
Add diluted samples/controls/standards to the microplate wells	50 µL
Incubate at 37°C for 2-6 min	
Add S-2732 (pre-heated at 37°C)	50 µL
Mix and add within 2 min Factor Xa (pre-heated at 37°C)	50 µL
Mix and incubate at +37°C for 120 sec.	
Stop reaction with acetic acid 20% or citric acid 2%	50 µL

Read the absorbance against water (see REAGENT 3) at 405 nm.
If possible, read and subtract the absorbance at 490 nm in order to compensate for differences in the material of the microplate wells.

Calibration

For the calibration of LMW Heparin or UF Heparin use a source of material which has been calibrated against an International Standard preparation.
For example: To prepare standards for 10 runs.
i. Dilute heparin with saline (0.9% NaCl) to obtain a working solution with a value of 100 IU/mL.
ii. Add 160 µL of the heparin working solution to 20.0 mL of normal plasma to obtain the heparin concentration of 0.8 IU/mL. Dilute according to the table below.

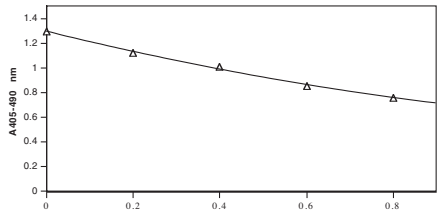
Standard IU/mL	Plasma with heparin 0.8 IU/mL mL	Normal plasma mL
0	-	4.0
0.2	1.0	3.0
0.4	2.0	2.0
0.6	3.0	1.0
0.8	4.0	-

These standards can be kept in aliquots at -20°C for 12 months.

Calculation

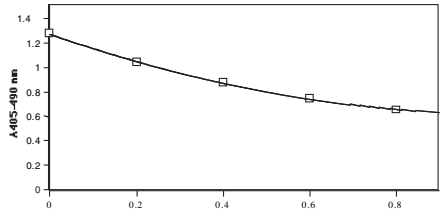
Microplate Method:
Plot the absorbance (A) for the standards against the concentration of heparin using an appropriate software program or on linear graph paper. Plot A on the Y-axis and IU/mL heparin on the X-axis. Connect the standard points with the best fitting second order polynomial line. Samples and controls are evaluated based on this standard curve.
Examples of typical standard curves (microplate method) are shown below:

Standard curve Unfractionated heparin.



IU/mL Unfractionated heparin

Standard curve LMW heparin.



IU/mL LMW heparin

Performance characteristics

Limitations/interfering substances
Heparin results are not affected by hemoglobin up to 200 mg/dl, triglycerides up to 600 mg/dl and bilirubin up to 12 mg/dl.
The presence of dextran sulfate reduces the influence from heparin antagonists, e.g. platelet factor 4 (PF4).[4]

Precision:
Microplate method. The data summarized below was obtained with the microplate method using unfractionated heparin (UFH) and low molecular weight heparin (LMWH).

Mean concentration	Within run C.V. (%)	Between run C.V. (%)	Total C.V. (%)
0.7 IU/mL UFH	2.8	1.2	2.8
0.4 IU/mL UFH	3.4	1.5	3.7
0.7 IU/mL LMWH	3.6	2.8	4.4
0.4 IU/mL LMWH	2.4	2.3	3.2

Examples of instrument-specific precision results obtained by or for Chromogenix are included in the instrument application sheets.
Each laboratory should establish their own precision data.

Correlation:
1. The COAMATIC® Heparin assay shows good correlation with COATEST® Heparin and COATEST® LMW Heparin/Heparin performed on the Cobas Mira instrument:

   COAMATIC® Heparin versus COATEST® Heparin (n = 112)
   IU/mL Heparin = +0.005 + 1.00 x Heparin, r = 0.97

   COAMATIC® Heparin versus COATEST® LMW Heparin/Heparin (n = 90)
   IU/mL Heparin = +0.002 + 1.04 x Heparin, r = 0.96

2. The COAMATIC® Heparin (performed on various Instruments) versus IL Test™ Heparin performed on the ACL 300 Instrument

Microplate			
	(n = 70)	IU/mL Heparin = +0.017 + 1.00x Heparin,	r = 0.97
Instruments			
Cobas Mira	(n = 87)	IU/mL Heparin = -0.012 + 1.04x Heparin,	r = 0.98
ACL 300†	(n = 62)	IU/mL Heparin = +0.004 + 1.03x Heparin,	r = 0.98
Futura	(n = 113)	IU/mL Heparin = +0.009 + 0.97x Heparin,	r = 0.97
MLA Electra 1600	(n = 80)	IU/mL Heparin = +0.004 + 1.01x Heparin,	r = 0.97
Thrombolyzer*	(n= 76)	IU/mL Heparin = +0.008 + 0.92x Heparin	r = 0.97
BCS*	(n = 30)	IU/mL Heparin = +0.008 + 0.93x Heparin,	r = 0.99
STA*	(n = 29)	IU/mL Heparin = +0.017 + 0.96x Heparin,	r = 0.99
Sysmex 6000	(n = 30)	IU/mL Heparin = +0.061 + 0.91x Heparin,	r = 0.99
AMAX	(n = 30)	IU/mL Heparin = +0.028 + 0.97x Heparin,	r = 0.99
AMGA*	(n = 30)	IU/mL Heparin = -0.061 + 1.02x Heparin,	r = 0.99
Hitachi 911	(n = 30)	IU/mL Heparin = +0.014 + 0.98x Heparin,	r = 0.99
Hitachi 917	(n = 30)	IU/mL Heparin = +0.021 + 1.00x Heparin,	r = 0.98

*NOTE: Instrument not available in all countries.
†NOTE: In the case of the ACL Hundred/Thousand Series, the assay is run as a two-stage method with the addition of antithrombin reagent. See the Instrument Application Sheet for specific information.

Recommended measuring range

For the microplate method the relationship between the heparin concentration and the pNA release, measured as absorbance at 405 nm, follows a second order polynomial function in the range of 0-1.5 IU/mL.

Sensitivity:

System	
Cobas Mira	mAbs / min per 1IU/mL Heparin 333 mAbs

Determinations/kit

Microplate: 200

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PORTUGUÊS - Revisão do folheto 12/2008

Para a revisao actual deste folheto informativo em Portugues, contacte o representante da Chromogenix da sua área.

PRECAUÇÕES E ADVERTÊNCIAS:
Evitar o contacto com a pele e os olhos (S 24/25).
Não deitar os resíduos nos esgotos (S 29).
Usar equipamento de protecção adequado (S 36).
Este reagente destina-se a utilização em diagnóstico ***in vitro.***

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SVENSK - Instick revision 12/2008

För aktuell revision av detta insticksblad på svenska ber vi Er att kontakta Chromogenix distributör.

FÖRSIKTIGHETSÅTGÄRDER OCH VARNINGAR:
Undvik kontakt med hud och ögon (S24/25).
Töm ej i slasken (S29).
Använd ändamålsenlig skyddsklädsel (S36).
Denna produkt är för ***in vitro*** diagnostiskt användande.

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DANSK - Metodeforskrift revision 12/2008

Venligst rekvirer den gældende udgave af metodeforskriften på dansk fra den lokale Chromogenix distributør.

ADVARSEL:
Undgå kontakt med hud og øjne (S24/25).
Må ikke hældes i laboratorievaskens afløb (S29).
Arbejd iført kittel og handsker (S36).
Dette produkt er til ***in vitro*** diagnostisk anvendelse.

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ΕΛΛΗΝΙΚΑ - Αναθεώρηση εσωκλείστου 12/2008

Για την τρέχουσα αναθεώρηση αυτού του εσωκλείστου στα Ελληνικά, παρακαλούμε επικοινωνήστε με τον τοπικό αντιπρόσωπο της CHROMOGENIX.

ΠΡΟΣΟΧΗ:
Αποφύγετε την επαφή με το δέρμα και τα μάτια (S24/S25).
Μην απορρίπτετε στην αποχέτευση (S29).
Φοράτε τα κατάλληλα προστατευτικά ενδύματα (S36).
Το προϊόν προορίζεται για διαγνωστική χρήση in vitro.

Bibliography / Literatur / Bibliografía / Bibliographie / Bibliografia /Bibliografia / Litteratur / Litteraturförteckning / Βιβλιογραφία

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4. National Committee for Clinical Laboratory Standards. Collection, transport and processing of blood specimens for coagulation testing and performance of coagulation assays, NCCLS Document H21-A2; vol 11 No. 23.
5. ÖDEGÅRD O R et al. Heparin cofactor activity measured with an amidolytic method. Thromb Res 6, 287-294 (1975).
6. TRANT H et al. Influence of heparin cofactor II (HCII) in the determination of antithrombin III (AT). Thromb Res 40, 571-576 (1985).
7. TOLLEFSEN D M. Laboratory diagnosis of antithrombin and heparin cofactor II deficiency. Semin Thromb Hemost 16, 162-168 (1990).
8. DEMERS C et al. An antithrombin III assay based on factor Xa inhibition provides a more reliable test to identify congenital antithrombin III deficiency than an assay based on thrombin inhibition. Thromb Haemost 69, 231-235 (1993).
9. BOHNER J et al. Thrombin-based antithrombin assays show overestimation of antithrombin III activity in patients on heparin therapy due to heparin cofactor II influence. Thromb Haemost 71, 280-283 (1994).

Symbols used / Verwendete Symbole / Símbolos utilizados / Symboles utilisés / Simboli impiegati / Símbolos utilizados / Anvendte symboler / Använda Symboler / Χρησιμοποιηθέντα σύμβολα

IVD	LOT					EC REP
In vitro diagnostic medical device	Batch code	Use by	Temperature limitation	Consult instructions for use	Manufacturer	Authorised representative
In-vitro Diagnostikum	Chargen-Bezeichnung	Verwendbar bis	Festgelegte Temperatur	Beilage beachten	Hergestellt von	Bevollmächtigter
De uso diagnóstico ***in vitro***	Identificación número de lote	Caducidad	Temperatura de Almacenamiento	Consultar la metódica	Fabricado por	Representante autorizado
Dispositif mèdical de diagnostic ***in vitro***	Désignation du lot	Utilisable jusqu'à	Températures limites de conservation	Lire le mode d'emploi	Fabricant	Mandataire
Per uso diagnostico ***in vitro***	Numero del lotto	Da utilizzare prima del	Limiti di temperatura	Vedere istruzioni per l'uso	Prodotto da	Rappresentanza autorizzata
Dispositivo médico para utilização em diagnóstico ***in vitro***	Número de lote	Data límite de utilização	Límite de temperatura	Consultar as instruções de utilização	Fabricado por	Representante autorizado
"in vitro" diagnostisk udstyr	Batch nr.	Anvendelse	Temperatur begrænsninger	Se vejledning for anvendelse	Producent	Leverandør
In vitro diagnostisk medicinsk produkt	Tillverkningskod	Användning	Temperatur gräns	Ta del av instruktionen före användning	Tillverkare	Auktoriserad representant
Προϊόν για διαγνωστική χρήση ***In vitro***	Αρ. Παρτίδας	Χρήση έως	Περιορισμοί θερμοκρασίας	Συμβουλευτήτε τις οδηγίες χρήσης	Κατασκευαστής	Εξουσιοδοτημένος αντιπρόσωπος

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