# Heparin - 0020009400

**ENGLISH - Insert revision 03/2005** CE

## Intended use

Automated chromogenic assay for the quantitative determination of unfractionated heparin (UFH) and low molecular weight heparin (LMWH) activity in human citrated plasma on the IL Coagulation Systems.

## Summary and principle

Heparin is the most frequently used antithrombotic drug. The biological activity of this sulphated glycosaminoglycan resides in its ability to accelerate (up to 2000-fold) the inhibitory effect of antithrombin on coagulation proteases. In recent years, it has been shown that LMWH, besides being as useful therapeutically as UFH, also has a longer half-life.
The Heparin kit is an assay based on a synthetic chromogenic substrate and on Factor Xa inactivation. Heparin levels in patient plasma are measured automatically on IL Coagulation Systems in two stages.

1. Heparin is analyzed as a complex with antithrombin present in the sample. The concentration of this complex is dependent on the availability of antithrombin. In order to obtain a more constant concentration of antithrombin, purified human antithrombin is added to the test plasma.[1] Factor Xa is added in excess and is neutralized by heparin-antithrombin complex.
2. Residual Factor Xa is quantified with a synthetic chromogenic substrate. The paranitroaniline released is monitored kinetically at 405 nm and is inversely proportional to the heparin level in the sample.[2]

Since different kinds of UF- and LMW- Heparins have their own specific anti-Factor Xa activity, the same kind of heparin as is used in the patient sample should also be used for calibrating the standard curve.[3,4]

## Composition

The **Heparin** kit consists of:

S **Chromogenic substrate** (Cat. No. 00020009410)**:** 1 x 4 mL vial of the lyophilized chromogenic substrate S-2765, N-α-Z-D-Arg-Gly-Arg-pNA·2HCl (3 mg/vial) and bulking agent.

E **Factor Xa reagent** (Cat. No. 00020009420)**:** 1 x 5 mL vial of a lyophilized preparation containing purified bovine Factor Xa (68 nkat/vial), Tris-buffer, EDTA, sodium chloride and bovine serum albumin.

A **Antithrombin** (Cat. No. 00020009430)**:** 1 x 3 mL vial of a lyophilized preparation containing human antithrombin (3 IU/vial), Tris-buffer, EDTA, sodium chloride and bovine serum albumin.

B **Buffer** (Cat. No. 00020009440)**:** 1 x 8 mL vial of a concentrated solution containing Tris-buffer, pH 8.4, EDTA, sodium chloride and detergent.

**PRECAUTIONS AND WARNINGS:**
The material in this product was tested with FDA cleared methods and found nonreactive for Hepatitis B surface Antigen (HBsAg), Anti-HCV and HIV antibodies. Handle as if potentially infectious.[5]

Avoid contact with skin and eyes (S 24/25). Do not empty into drains (S 29). Wear suitable protective clothing (S 36).
All animal products should be treated as potentially infectious.

This product is For *in vitro* Diagnostic Use.

## Preparation

**Chromogenic substrate:** Dissolve the vial contents with 4 mL of NCCLS Type II water or equivalent.[6] Replace the stopper and swirl gently. Make sure of the complete reconstitution of the product. Keep the substrate at 15-25°C for 30 minutes and invert to mix before use.

**Factor Xa reagent:** Dissolve the vial contents with 5 mL of NCCLS Type II water or equivalent.[6] Replace the stopper and swirl gently. Make sure of the complete reconstitution of product. Keep the reagent at 15-25°C for 30 minutes and invert to mix before use.

**Antithrombin:** Dissolve the vial contents with 3 mL of NCCLS Type II water or equivalent.[6] Replace the stopper and swirl gently. Make sure of the complete reconstitution of product. Keep the reagent at 15-25°C for 30 minutes and invert to mix before use.
**Buffer:** Dilute the necessary quantity of concentrated buffer 1:10 (1+9) with NCCLS Type II water or equivalent.[6] Mix before use**.**
**Working buffer:** To 24 mL of diluted buffer add 1 mL of reconstituted antithrombin reagent.

**Note:** An instant opalescence will occur in the lyophilized reagents but it will fade away within 2 minutes.

## Reagent storage and stability

Unopened reagents are stable until the expiration date shown on the vial when stored at 2-8°C.

**Chromogenic substrate -** Stability after reconstitution: 7 days at 15°C, 3 months at 2-8°C in the original vial or 48 hours at 15°C on the ACL Futura®/ACL Advance Systems and ACL TOP™.

**Factor Xa reagent -** Stability after reconstitution: 7 days at 15°C, 3 months at 2-8°C in the original vial or 48 hours at 15°C on the ACL Futura/ACL Advance Systems and ACL TOP.

**Antithrombin -** Stability after reconstitution: 3 months at 2-8°C in the original vial.

**Buffer -** Opened reagent is stable 3 months at 2-8°C.

**Working buffer -** Stability after preparation: 7 days at 15°C and 2-8°C in a closed container or 48 hours at 15°C on the ACL Futura/ACL Advance Systems and ACL TOP.

For optimal stability remove reagents from the system and store them at 2-8°C in the original vial.

## Instrument/test procedures

Refer to the appropriate IL instrument's Operator's Manual and/or Application Manual for the complete assay procedure instructions.

**Note:** A cleaning cycle is recommended after running the Heparin assay on all IL Coagulation Systems.

## Specimen collection and preparation

Nine parts of freshly drawn venous blood are collected into one part trisodium citrate. Refer to NCCLS Document H21-A4 for further instructions on specimen collection, handling and storage.[7]

## Standardization

For preparation of standards use the same heparin as used for patient therapy. Refer to the instrument's Operator's Manual for complete instructions on their preparation.

## Additional reagent and control plasmas

The following are not supplied with the kit and must be purchased separately.

| | Americas and Pacific Rim Cat. No. | Europe Cat No. |
|---|---|---|
| Calibration plasma | 0020000000 | 0008467300 |
| Low Heparin control* | 20004000 | N/A |
| High Heparin control* | 20004100 | N/A |
| Cleaning solution | 0009831700 | 0009831700 |
| Cleaning agent | 0009832700 | 0009832700 |

***Note**: Not for use with low molecular weight heparin (LMWH) assays.

## Quality control

Two levels of controls are recommended for a complete quality control program.[8] Low* and High* Heparin control are designed for this program.

Each laboratory should establish its own mean and standard deviation and should establish a quality control program to monitor laboratory testing. Controls should be analyzed at least once every 8 hour shift in accordance with good laboratory practice. Refer to the instrument's Operator's Manual for additional information. Refer to Westgard *et al* for identification and resolution for out-of-control situations.[9]

## Results

Heparin results are reported in U/mL. Refer to the instrument's Operator's Manual for additional information.

## Limitations/interfering substances

Heparin results on the ACL and ACL Futura/ACL Advance Systems are not affected by hemoglobin up to 200 mg/dL, bilirubin up to 20 mg/dL and triglycerides up to 700 mg/dL.

Heparin results on the ACL TOP are not affected by hemoglobin up to 375 mg/dL, bilirubin up to 25 mg/dL and triglycerides up to 1630 mg/dL.

## Expected values

To obtain an optimal effect with minimum risk of bleeding or thromboembolic complications the heparin activity should be in the range recommended by the heparin manufacturer.[10]

## Performance characteristics

**Precision:**
Within run and total (run to run and day to day) precision was assessed over multiple runs using two levels of sample of both the 4th International Standard for heparin (UFH) and Fragmin® (LMWH).

| ACL® Family | Mean (U/mL) | CV % (Within run) | CV % (Total) |
|---|---|---|---|
| UFH | 0.77 | 1.84 | 2.18 |
| UFH | 0.23 | 7.76 | 8.23 |
| LMWH | 0.79 | 2.68 | 3.09 |
| LMWH | 0.23 | 7.99 | 9.69 |
| **ACL Futura/ACL Advance** | **Mean (U/mL)** | **CV % (Within run)** | **CV % (Total)** |
| UFH | 0.79 | 3.0 | 6.6 |
| UFH | 0.52 | 5.7 | 7.3 |
| UFH | 0.26 | 9.1 | 10.0 |
| LMWH | 0.76 | 4.1 | 4.5 |
| LMWH | 0.42 | 6.2 | 7.9 |
| LMWH | 0.22 | 10.7 | 11.9 |
| **ACL TOP** | **Mean (U/mL)** | **CV % (Within run)** | **CV % (Total)** |
| UFH | 0.82 | 1.8 | 6.4 |
| UFH | 0.53 | 3.4 | 4.3 |
| UFH | 0.25 | 4.6 | 8.5 |
| LMWH | 0.86 | 2.4 | 4.4 |
| LMWH | 0.49 | 6.1 | 8.2 |
| LMWH | 0.25 | 5.7 | 11.3 |

**Correlation:**

| System | slope | intercept | r | Reference method |
|---|---|---|---|---|
| ACL Family | 0.968 | 0.014 | 0.988 | IL Heparin (Xa) |
| ACL Futura/ACL Advance | 0.944 | 0.035 | 0.989 | IL Heparin (Xa) |
| ACL TOP | 1.012 | -0.005 | 0.992 | HemosIL Heparin (Xa) on ACL Advance |

The precision and correlation results were obtained using specific lots of reagent and controls.

**Linearity:**

| System | |
|---|---|
| ACL Family and ACL Futura/ACL Advance | 0 - 1.0 U/mL |
| ACL TOP | 0 - 1.1 U/mL |

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


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

| IVD | LOT | | | | CONTROL | | | EC REP |
|---|---|---|---|---|---|---|---|---|
| *In vitro* diagnostic medical device | Batch code | Use by | Temperature limitation | Consult instructions for use | Control | Biological risks | Manufacturer | Authorised representative |
| *In-vitro* Diagnostikum | Chargen-Bezeichnung | Verwendbar bis | Festgelegte Temperatur | Beilage beachten | Kontrollen | Biologisches Risiko | Hergestellt von | Bevollmächtigter |
| De uso diagnóstico *in vitro* | Identificación número de lote | Caducidad | Temperatura de Almacenamiento | Consultar la metódica | Control | Riesgo biológico | 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 | Contrôle | Risque biologique | Fabricant | Mandataire |
| Per uso diagnostico *in vitro* | Numero del lotto | Da utilizzare prima del | Limiti di temperatura | Vedere istruzioni per l'uso | Controllo | Rischio biologico | 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 | Controlo | Risco biológico | Fabricado por | Representante autorizado |
| "in vitro" diagnostisk udstyr | Batch nr. | Anvendelse | Temperatur begrænsninger | Se vejledning for anvendelse | Kontrol | Miljø oplysninger | Producent | Leverandør |
| *In vitro* diagnostisk medicinsk produkt | Tillverkningskod | Användning | Temperatur gräns | Ta del av instruktionen före användning | Kontroll | Biologiska risker | Tillverkare | Auktoriserad representant |
| Προϊόν για διαγνωστική χρήση *In vitro* | Αρ. Παρτίδας | Χρήση έως | Περιορισμοί θερμοκρασίας | Συμβουλευτήτε τις οδηγίες χρήσης | Υλικό ποιοτικού ελέγχου | Βιολογικοί κίνδυνοι | Κατασκευαστής | Εξουσιοδοτημένος αντιπρόσωπος |

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