



ANTI-XA HEPARIN KIT

For Research Use Only

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INTENDED USE

The r² Diagnostics Anti-Xa Heparin assay kit is designed for the chromogenic measurement of the anti-factor Xa activity in samples containing Unfractionated heparin (UFH), Low Molecular Weight heparin (LMWH), and related molecules with factor Xa inhibitor activity. These kits are designed and optimized for activity measurements with buffer or plasma samples. The Anti-Xa kit is ideally suited for research, industrial, and pharmacopeial test applications.

SUMMARY AND PRINCIPLE

Factor Xa serves a key role in the classical coagulation cascade where it serves as part of the Xase complex that converts prothrombin to thrombin. Unfractionated Heparin (UFH) and Low Molecular Weight Heparin (LMWH) inhibit blood coagulation via initial binding of these molecules to Antithrombin III (ATIII). The ATIII-heparin complex inhibits Factor Xa, as well as thrombin (Factor IIa). In buffer, this same “Anti-Xa” activity of heparin can be followed enzymatically by means of a chromogenic peptide substrate which generates a chromophore (405 nm), whose measure is inversely proportional to the initial amount of heparin present in the test sample. The para-nitroaniline (pNA) chromophore generated by the residual factor Xa can be measured either kinetically or by an acid-stopped end-point method.

Schematically, the procedure is carried out as follows:

ATIII + Heparin	→ [ATIII-Heparin]
[ATIII-Heparin] + Xa (known excess)	→ Xa (residual) + Xa-ATIII-Heparin (inactive)
Xa (residual) + Xa Substrate (colorless)	→ pNA chromophore (405 nm) + peptide

REAGENTS AND COMPONENTS

- Factor Xa, bovine, 25 µg/ vial** **2 vials**
Lyophilized purified bovine factor Xa and bovine albumin.
Reconstitute in 10mL chromogenic assay buffer according to specific protocol instructions.
- Xa Substrate, 5 mg/ vial** **2 vials**
Lyophilized chromogenic substrate Z-D-Arg-Gly-Arg-pNA · 2HCl
Reconstitute in 5mL DI water according to specific protocol instructions.
- Antithrombin, human 5 IU/ vial** **2 vials**
Lyophilized purified human antithrombin and bovine albumin.
Reconstitute in 5mL chromogenic assay buffer according to specific protocol instructions.
- Chromogenic Assay buffer** **1 bottle**
Use to reconstitute Antithrombin and Factor Xa reagent vials in the kit,

Note: The Antithrombin, human 5 IU was tested and found negative for HIV, Hepatitis B, and Hepatitis C. However, as with biohazardous samples, handle all reagents as potentially infectious and follow appropriate safety procedures.

MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

- Calibrated pipettes
- Acetic Acid, 20% (acid-stopped methods)
- Spectrophotometer, microplate reader, or automated hemostasis instrument with 405 nm wavelength reading capability
- Water-bath incubator (37°C)
- International (WHO) or pharmacopeial (USP, EP) Heparin or LMW-heparin Reference Standard
- Timer or stop watch

STORAGE AND STABILITY

The kit may be used up to the expiration date given on the label when stored unopened at 2 – 8°C.

Stability of the reagents after reconstitution:

- 2-8°C 1 week
- 15-19°C 48 hours