



ANTI-IIa HEPARIN KIT

For Research Use Only

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INTENDED USE

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

SUMMARY AND PRINCIPLE

Thrombin (Factor IIa) is the terminal enzyme in the classical coagulation cascade, where it plays roles in both the fibrin deposition and platelet activation processes of blood coagulation. Unfractionated Heparin (UFH) and Low Molecular Weight Heparin (LMW-H) inhibit blood coagulation via initial binding of these molecules to Antithrombin (AT), followed subsequently by the AT-heparin complex inhibiting Factor IIa. In buffer, this same "Anti-IIa" activity of heparin can be followed enzymatically by means of a chromogenic peptide substrate which, upon hydrolysis by residual factor IIa, provides a chromophore (405 nm) whose measure is inversely proportional to the initial amount of heparin present in the test sample. The paranitroaniline (pNA) chromophore generated by the residual factor IIa 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] + IIa (known excess)	→ IIa (residual) + IIa-ATIII-Heparin (inactive)
IIa (residual) + IIa Substrate (colorless)	→ pNA chromophore (405 nm) + peptide

REAGENTS AND COMPONENTS

- 1. Thrombin, Human, 40 IU/ vial** **2 vials**
Lyophilized purified human alpha thrombin and bovine albumin.
Reconstitute in 10mL chromogenic assay buffer according to specific protocol instructions.
- 2. IIa Substrate, 6.25 mg/ vial** **2 vials**
Lyophilized chromogenic substrate H-D-Phe-Pip-Arg-pNA. 2HCl
Reconstitute in 5mL DI water according to specific protocol instructions.
- 3. Antithrombin, human 2.5IU/ vial** **2 vials**
Lyophilized purified human antithrombin and bovine albumin.
Reconstitute in 5mL chromogenic assay buffer according to specific protocol instructions.
- 4. Chromogenic Assay Buffer** **1 bottle**
Use to reconstitute Antithrombin III and Thrombin reagent vials in the kit.

Note: The Thrombin, Human 40 IU & Antithrombin, human 2.5 IU were 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 retest 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