



Provision Kinetics, Inc.  
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## ANTI-IIA HEPARIN KIT

Item #: IIAI-200 or IIAI-100

**For Research Use Only**

### INTENDED USE

The Kinetichrome™ Heparin Anti-IIa kit is designed for the chromogenic measurement of the anti-factor IIa (thrombin) activity in samples containing heparin, Low Molecular Weight heparin (LMW-heparin), and related molecules with thrombin inhibitor activity. A distinctive feature of the Kinetichrome™ product line is that these kits are designed and optimized for activity measurements with buffer as the reaction medium, rather than plasma. Thus, the Kinetichrome™ Anti-IIa kit is ideally suited for research, industrial, and pharmacopeial test applications where buffer is the specified test medium.

### 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 paranitroanaline (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:

AT + heparin → [AT-heparin]

[AT-heparin] + IIa (known excess) → IIa (residual) + IIa-AT-heparin (inactive)

IIa (residual) + IIa Substrate (colorless) → pNA chromophore (405 nm) + peptide

### REAGENTS AND COMPONENTS

- 1. Thrombin, human 40 IU** **2 vials**  
Lyophilized purified human thrombin (factor IIa) and bovine albumin.  
Reconstitute in buffer according to specific protocol instructions.
- 2. IIa Substrate, 6.25 mg** **2 vials**  
Lyophilized chromogenic substrate H-D-Phe-Pip-Arg-pNA·2HCl  
Reconstitute in water according to specific protocol instructions.
- 3. Antithrombin, human 2.5 IU** **2 vials**  
Lyophilized purified human antithrombin and bovine albumin.  
Reconstitute in buffer according to specific protocol instructions.

**Note:** The Thrombin, human 40 IU, and Antithrombin, human 2.5 IU were tested and found negative for HIV, Hepatitis B, and Hepatitis C. However, no test can rule out the presence of these infectious diseases. Handle all reagents as potentially infectious.

### MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

- Calibrated pipettes (75 to 9000 µl)
- 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