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U. S. A.

ANTI-XA HEPARIN KIT

Item #: XAI-200 or XAI-100

For Research Use Only

INTENDED USE

The Kinetichrome™ Heparin Anti-Xa kit is designed for the chromogenic measurement of the anti-factor Xa activity in samples containing heparin, Low Molecular Weight heparin (LMW-heparin), and related molecules with factor Xa inhibitor activity. A distinctive feature of the Kinetichome™ 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-Xa kit is ideally suited for research, industrial, and pharmacopeial test applications where buffer is the specified or preferred test medium.

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 pro-thrombin to thrombin. 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 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 paranitroanaline (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:

AT + heparin $\rightarrow$ [AT-heparin]

[AT-heparin] + Xa (known excess) $\rightarrow$ Xa (residual) + Xa-AT-heparin (inactive)

Xa (residual) + Xa Substrate (colorless) $\rightarrow$ pNA chromophore (405 nm) + peptide

REAGENTS AND COMPONENTS

1. Factor Xa, bovine, 25 µg **2 vials**
Lyophilized purified bovine factor Xa and bovine albumin.
Reconstitute in buffer according to specific protocol instructions.

2. Xa Substrate, 6.25 mg **2 vials**
Lyophilized chromogenic substrate CH_3OCO-D-CHA-Gly-Arg-pNA-AcOH
Reconstitute in water according to specific protocol instructions.

3. Antithrombin, human 5 IU **2 vials**
Lyophilized purified human antithrombin and bovine albumin.
Reconstitute in buffer according to specific protocol instructions.

Note: The Antithrombin, human 5 IU was tested and found negative for HIV, Hepatitis B, and Hepatitis C. However, no test can completely 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