

Antithrombin Assay

Antithrombin activity is measured by level of inhibition of Factor Xa

Materials

1. Versa Max microtiter plate reader (Molecular Devices, CA)
2. 96-well microtiter plate
3. Citrated plasma samples
4. Normal mouse plasma (NMP)
5. HN/BSA
6. Factor Xa/heparin reagent
7. 1.25 mg/mL chromogenic substrate S-2765

Procedure

1. Plasma samples may be diluted to 1:40 - 1:200 in HN/BSA
2. Standard curve is prepared with each plate by diluting NMP 1:20 to 1:640 in HN/BSA, analyzed simultaneously on the sample plate
3. Plate wells are loaded as follows:

Sample dilutions, in duplicate	40uL	37°C	3min	
Factor Xa/Heparin reagent	40uL			
Chromogenic substrate	40uL			Color development

4. Plate is read at 405 nm
5. Absorbance are converted to %NMP Antithrombin using the standard curve