

uPA assay kit

Rat uPA total antigen ELISA assay

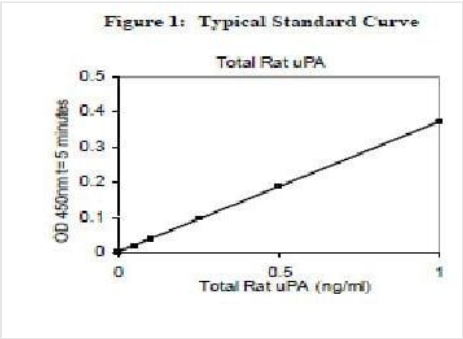
CATALOG #
AAA71832

PRODUCT SYNONYMS

uPA; Rat uPA total antigen ELISA assay; Rat uPA Total Antigen ELISA; uPA assay kit

PRODUCT SPECIFICATIONS

Procedural Notes	1. Use aseptic technique when opening and dispensing reagents. 2. This kit is designed to work properly as provided and instructed. Additions, deletions, or substitutions to the procedure or reagents are not recommended, as they may be detrimental to the assay. 3. Exercise universal precautions during the performance or handling of this kit or any component contained therein.
Sample Collection and Preparation	Collect 9 volumes of blood in 1 volume of 0.1 M trisodium citrate or acidified citrate, preferably using Stabilyte evacuated vials. Immediately after collection of blood, samples must be centrifuged at 3000 x g for 15 minutes. The plasma must be transferred to a clean plastic tube and must be stored on ice prior to analysis. The uPA activity samples collected in the Stabilyte media are stable for up to 24 hours or stored at -20 degree C for up to one month and thawed three times without loss of uPA activity. The assay measures total uPA in the 0.05-10 ng/ml range. Samples giving uPA levels above 10 ng/ml should be diluted in plasma devoid of uPA.
Reagent Preparation	1. 10x Wash Buffer: Dilute the 50 mL of concentrate to 1x with 450 mL of DI water prior to use. 2. TBS Buffer: 0.1 M Tris, 0.15 M NaCl, pH 7. 4. 3. 3% BSA Blocking Buffer: 3% BSA in TBS Buffer. 4. Biotinylated PAI-1: Reconstitute with 3% BSA Blocking Buffer as directed on the vial and vortex gently to mix. Prepare immediately prior to use. 5. Primary Antibody: Reconstitute with 3% BSA Blocking Buffer as directed on the vial and vortex gently to mix. Prepare immediately prior to use. 6. Secondary Antibody: Dilute with 3% BSA Blocking Buffer as directed on the vial and vortex gently to mix. Prepare immediately prior to use.
Standard Preparation	Reconstitute the Standard as directed on the vial to give a 100 ng/mL Standard Stock Solution. Do not prepare standards until you are ready to apply them to the plate.
Preparation and Storage	1. Store this kit and its components at 4 degree C until use. 2. The reconstituted Standards and Primary Antibody may be stored at -70C for later use. DO NOT freeze/thaw the Standards or Primary Antibody more than once.



Scheme I:

	1	2	3	4	5	6	7	8	9	10	11	12
A	S ₈	S ₈	U ₁	U ₁	U ₉	U ₉	U ₁₇	U ₁₇	U ₂₅	U ₂₅	U ₃₃	U ₃₃
B	S ₇	S ₇	U ₂	U ₂	U ₁₀	U ₁₀	U ₁₈	U ₁₈	U ₂₆	U ₂₆	U ₃₄	U ₃₄
C	S ₆	S ₆	U ₃	U ₃	U ₁₁	U ₁₁	U ₁₉	U ₁₉	U ₂₇	U ₂₇	U ₃₅	U ₃₅
D	S ₅	S ₅	U ₄	U ₄	U ₁₂	U ₁₂	U ₂₀	U ₂₀	U ₂₈	U ₂₈	U ₃₆	U ₃₆
E	S ₄	S ₄	U ₅	U ₅	U ₁₃	U ₁₃	U ₂₁	U ₂₁	U ₂₉	U ₂₉	U ₃₇	U ₃₇
F	S ₃	S ₃	U ₆	U ₆	U ₁₄	U ₁₄	U ₂₂	U ₂₂	U ₃₀	U ₃₀	U ₃₈	U ₃₈
G	S ₂	S ₂	U ₇	U ₇	U ₁₅	U ₁₅	U ₂₃	U ₂₃	U ₃₁	U ₃₁	U ₃₉	U ₃₉
H	S ₁	S ₁	U ₈	U ₈	U ₁₆	U ₁₆	U ₂₄	U ₂₄	U ₃₂	U ₃₂	U ₄₀	U ₄₀

Application Data

Table 1: Preparation of Standard Curve

Standard	uPA Concentration (ng/mL)	Blocking Buffer (µL)	Transfer Volume (µL)	Transfer Source	Final Volume (µL)
S ₈	10	900	100	Stock	900
S ₇	5	500	500	S ₈	600
S ₆	2	600	400	S ₇	500
S ₅	1	500	500	S ₆	500
S ₄	0.5	500	500	S ₅	500
S ₃	0.25	500	500	S ₄	600
S ₂	0.1	600	400	S ₃	500
S ₁	0.05	500	500	S ₂	1,000

Application Data

ADDITIONAL INFORMATION

Related Product Information for uPA assay kit	Introduction: Rat uPA total antigen assay is intended for the quantitative determination of total plasminogen activator antigen in rat plasma. Urokinase plasminogen activator (uPA) is a serine protease that activates plasminogen to plasmin in the blood fibrinolytic system. It is also implicated in events related to cell invasion/migration (3). Principle of assay: Rat uPA will bind to the capture antibody coated on the microtiter plate. Free, latent, and complexed enzyme will react with the capture antibody on the plate. After appropriate washing steps, polyclonal antirat uPA primary antibody binds to the captured enzyme. Excess antibody is washed away and bound polyclonal antibody is then reacted with the secondary antibody conjugated to horseradish peroxidase. TMB substrate is used for color development at 450 nm. A standard calibration curve is prepared along with the samples to be measured using dilutions of uPA.
References	1. Declerck, P.J., et al.; (1995) Thromb Haemostas: 74(5): 1305-9 2. Singh, I., et al.; (2003) Circulation: 107(6): 869-875 3. Kj��ller, L.; (2002) Biol. Chem.: 383: 5-19 4. Yang, Y.H., et al.; (2001) J. Immunol.: 167(2): 1047-52

For Research Use Only. Not for use in diagnostic or therapeutic procedures. Small volumes of product may become entrapped in the seal of the vial during shipment and storage; if necessary, briefly centrifuge the vial on a tabletop centrifuge to dislodge any liquid in the cap.