

uPA assay kit

Rat uPA activity ELISA assay

CATALOG #
AAA71831

PRODUCT SYNONYMS

uPA; Rat uPA activity ELISA assay; Rat uPA Active 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. It is important to ensure a platelet free preparation as platelets can release PAI-1, which in turn could potentially form a complex with uPA. 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.
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 the standards until you are ready to apply them to the plate.

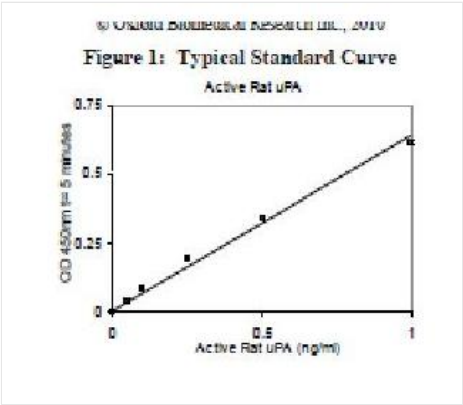
Table 1: Preparation of Standard Curve

Standard			
uPA			
Concentration			
(ng/mL)			
Blocking			
Buffer			
(μL)			
Transfer			
Volume			
(μL)			
Transfer			
Source			
Final			
Volume			
(μL)			
S9	10	900	100 Stock Vial 500
S8	5	500	500 S9 600
S7	2	600	400 S8 500
S6	1	500	500 S7 500
S5	0.5	500	500 S6 500
S4	0.25	500	500 S5 600
S3	0.1	600	400 S4 500
S2	0.05	500	500 S3 500
S1	0.025	500	500 S2 1,000
B0	0	500	--- --- 500

Preparation and Storage

1. Store this kit and its components at 4 degree C until use.
2. The reconstituted Standard and Primary Antibody may be stored at -70C for later use.
- DO NOT
- freeze/thaw the Standard or Primary Antibody more than once.

IMAGES



Application Data

Scheme 1:

	1	2	3	4	5	6	7	8	9	10	11	12
A	S9	S8	S7	S6	S5	S4	S3	S2	S1	B0	U1	U2
B	S9	S8	S7	S6	S5	S4	S3	S2	S1	B0	U1	U2
C	U3	U4	U5	U6	U7	U8	U9	U10	U11	U12	U13	U14
D	U3	U4	U5	U6	U7	U8	U9	U10	U11	U12	U13	U14
E	U15	U16	U17	U18	U19	U20	U21	U22	U23	U24	U25	U26
F	U15	U16	U17	U18	U19	U20	U21	U22	U23	U24	U25	U26
G	U27	U28	U29	U30	U31	U32	U33	U34	U35	U36	U37	U38
H	U27	U28	U29	U30	U31	U32	U33	U34	U35	U36	U37	U38

Application Data

ADDITIONAL INFORMATION

Related Product Information for uPA assay kit	Introduction: This rat urokinase plasminogen activator activity assay is for the quantitative determination of active plasminogen activator in rat plasma. Urokinase plasminogen activator (uPA) is a serine 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: Functionally active uPA will bind to the biotinylated human PAI-1 coated on the microtiter plate. Only free active enzyme will react with the PAI-1 on the plate. Inactive or complexed enzyme will not be detected. After appropriate washing steps, monoclonal mouse anti-rat uPA primary antibody binds to the captured enzyme. Excess antibody is washed away, and bound monoclonal 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.

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Online datasheet: <https://stage.aaabiotech.com/assay-kits/upa/71831>