

## Rat Urokinase Plasminogen Activator (uPA) Active Antigen ELISA

For Research Use Only

### 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).

### PRINCIPLES OF PROCEDURE

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.

### MATERIALS PROVIDED

Component	Contents	Quantity	Storage	Cat. No.
<b>Coated Plate</b>	Avidin coated 96-well plate	1 plate	4°C	UP41a
<b>Standard</b>	Rat uPA activity standard	1 vial	4°C	UP41b
<b>Biotinylated PAI-1</b>	Biotinylated PAI-1 (lyophilized)	1 vial	4°C	UP41c
<b>Primary Antibody</b>	Anti-rat uPA polyclonal antibody (lyophilized)	1 vial	4°C	UP41d
<b>Wash Buffer</b>	10x solution for washing plate	50 mL	4°C	UP41e
<b>Substrate</b>	TMB Substrate	10 mL	4°C	UP41f
<b>Secondary Antibody</b>	Anti-rabbit HRP conjugated antibody	1 vial	4°C	UP41g
<b>10x TBS Solution</b>	Tris Buffered Saline pH 7.4	5 mL	4°C	UP41h

### MATERIALS NEEDED BUT NOT PROVIDED

1. Pipettes covering 0-10  $\mu$ l and 200-1000  $\mu$ l and tips
2. 12-channel pipette covering 30-300  $\mu$ l
3. 1 N H<sub>2</sub>SO<sub>4</sub>
4. DI water
5. Microtiter plate spectrophotometer with a 450 nm filter
6. Microtiter plate shaker with uniform horizontally circular movement up to 300 rpm

### STORAGE CONDITIONS

1. Store this kit and its components at 4°C until use.

- The reconstituted Standard and Primary Antibody may be stored at  $-70^{\circ}\text{C}$  for later use. **DO NOT** freeze/thaw the Standard or Primary Antibody more than once.

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## PROCEDURAL NOTES

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- Use aseptic technique when opening and dispensing reagents.
- 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.
- Exercise universal precautions during the performance or handling of this kit or any component contained therein.

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## SAMPLE COLLECTION AND PREPARATION

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Collect 9 volumes of blood in 1 volume of 0.1 M trisodium citrate or acidified citrate, preferably using Stabilyte™ evacuated vials (Biopool, cat# 102080). Immediately after collection of blood, samples must be centrifuged at  $3000 \times g$  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  $-20^{\circ}\text{C}$  for up to one month and thawed three times without loss of uPA activity.

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## REAGENT PREPARATION

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- 10x Wash Buffer:** Dilute the 50 mL of concentrate to 1x with 450 mL of DI water prior to use.
- TBS Buffer:** 0.1 M Tris, 0.15 M NaCl, pH 7.4.
- 3% BSA Blocking Buffer:** 3% BSA in TBS Buffer.
- Biotinylated PAI-1:** Reconstitute with 3% BSA Blocking Buffer as directed on the vial and vortex gently to mix. Prepare immediately prior to use.
- Primary Antibody:** Reconstitute with 3% BSA Blocking Buffer as directed on the vial and vortex gently to mix. Prepare immediately prior to use.
- Secondary Antibody:** Dilute with 3% BSA Blocking Buffer as directed on the vial and vortex gently to mix. Prepare immediately prior to use.

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## SANDARD PREPARATION

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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 ( $\mu\text{L}$ )	Transfer Volume ( $\mu\text{L}$ )	Transfer Source	Final Volume ( $\mu\text{L}$ )
S <sub>9</sub>	10	900	100	Stock Vial	500
S <sub>8</sub>	5	500	500	S <sub>9</sub>	600
S <sub>7</sub>	2	600	400	S <sub>8</sub>	500
S <sub>6</sub>	1	500	500	S <sub>7</sub>	500
S <sub>5</sub>	0.5	500	500	S <sub>6</sub>	500
S <sub>4</sub>	0.25	500	500	S <sub>5</sub>	600
S <sub>3</sub>	0.1	600	400	S <sub>4</sub>	500
S <sub>2</sub>	0.05	500	500	S <sub>3</sub>	500
S <sub>1</sub>	0.025	500	500	S <sub>2</sub>	1,000
B <sub>0</sub>	0	500	---	---	500

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## ASSAY PROCEDURE

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1. Add 100  $\mu$ L of reconstituted Biotinylated PAI-1 to all of the wells. Shake the plate at 300 rpm for 30 minutes at room temperature (RT).
2. Wash the plate 3 times according to the following wash procedure:
  - a. Remove the contents of each well by inversion of the plate.
  - b. Tap out the remaining contents of the plate onto a lint free paper towel.
  - c. Add 300  $\mu$ L of 1x Wash Buffer.
  - d. Let stand for 2-3 minutes.
  - e. Repeat procedure two more times, then proceed to step “f”.
  - f. Remove the contents of each well by inversion of plate into an appropriate disposal device.
  - g. Tap out the remaining contents of the plate onto a lint free paper towel, then proceed to step 3.
3. Add 100  $\mu$ L of the Standards and unknowns to the wells in duplicate.
 

**NOTE:** If the pH of a sample is lower than 6.0, add 30  $\mu$ L of the 10x TBS Solution to the well prior to adding the sample. This step is unnecessary if the pH is neutral.

For a suggested plate layout, see Scheme I below. Shake the plate at 300 rpm for 30 minutes at RT.
4. Wash the plate three times as in step 2.
5. Add 100  $\mu$ L of the Primary Antibody to each well. Shake the plate at 300 rpm for 30 minutes at RT.
6. Wash the plate three times as in step 2.
7. Add 100  $\mu$ L of the Secondary Antibody to each well. Shake the plate at 300rpm for 30 minutes at RT.
8. Wash the plate three times as in step 2.
9. Add 100  $\mu$ L of TMB Substrate to each well. Shake the plate at 300 rpm for 10-20 minutes at RT.
10. Stop the reaction by adding 50  $\mu$ L of 1N H<sub>2</sub>SO<sub>4</sub> to each well and read the plate at 450 nm.

### Scheme I:

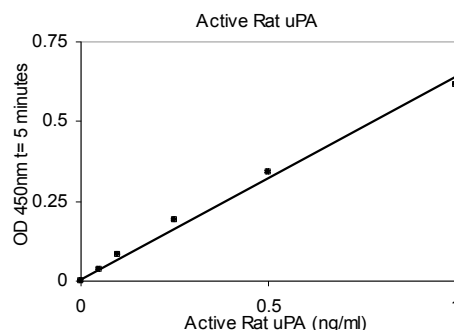
	1	2	3	4	5	6	7	8	9	10	11	12
A	S9	S8	S7	S6	S5	S4	S3	S2	S1	B0	U1	U2
B	S9	S8	S7	S6	S5	S4	S3	S2	S1	B0	U1	U2
C	U3	U4	U5	U6	U7	U8	U9	U10	U11	U12	U13	U14
D	U3	U4	U5	U6	U7	U8	U9	U10	U11	U12	U13	U14
E	U15	U16	U17	U18	U19	U20	U21	U22	U23	U24	U25	U26
F	U15	U16	U17	U18	U19	U20	U21	U22	U23	U24	U25	U26
G	U27	U28	U29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

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## CALCULATIONS

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1. Plot the A<sub>450</sub> against the concentration of uPA in the standards.
2. Fit a straight line through the points using a linear fit procedure.
3. Calculate the uPA concentrations in the unknowns using the equation generated by the standard curve.

**Figure 1: Typical Standard Curve**

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**EXPECTED VALUES**

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Abnormalities in uPA levels have been reported in the following conditions:

- Venous Thrombosis: Low levels of uPA are associated with clot formation (2).
- Inflammatory Disease: Low levels of uPA may aggravate this condition (4).

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**REFERENCES**

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3. Kjølner, L.; (2002) *Biol. Chem.*: **383**: 5-19
4. Yang, Y.H., *et al.*; (2001) *J. Immunol.*: **167(2)**: 1047-52

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