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.
Coated Plate	Avidin coated 96-well plate	1 plate	4°C	UP41a
Standard	Rat uPA activity standard	1 vial	4°C	UP41b
Biotinylated PAI-1	Biotinylated PAI-1 (lyophilized)	1 vial	4°C	UP41c
Primary Antibody	Anti-rat uPA polyclonal antibody (lyophilized)	1 vial	4°C	UP41d
Wash Buffer	10x solution for washing plate	50 mL	4°C	UP41e
Substrate	TMB Substrate	10 mL	4°C	UP41f
Secondary Antibody	Anti-rabbit HRP conjugated antibody	1 vial	4°C	UP41g
10x TBS Solution	Tris Buffered Saline pH 7.4	5 mL	4°C	UP41h

MATERIALS NEEDED BUT NOT PROVIDED

1. Pipettes covering 0-10 μl and 200-1000 μl and tips
2. 12-channel pipette covering 30-300 μl
3. 1 N H_2SO_4
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.
2. The reconstituted Standard and Primary Antibody may be stored at -70°C for later use. **DO NOT** freeze/thaw the Standard or Primary Antibody more than once.

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 (Biopool, cat# 102080). 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°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.

SANDARD 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)
S_9	10	900	100	Stock Vial	500
S_8	5	500	500	S_9	600
S_7	2	600	400	S_8	500
S_6	1	500	500	S_7	500
S_5	0.5	500	500	S_6	500
S_4	0.25	500	500	S_5	600
S_3	0.1	600	400	S_4	500
S_2	0.05	500	500	S_3	500
S_1	0.025	500	500	S_2	1,000
B_0	0	500	---	---	500

ASSAY PROCEDURE

1. Add 100 μl 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 μL 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 μl of the Standards and unknowns to the wells in duplicate.
   NOTE: If the pH of a sample is lower than 6.0, add 30 μL 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 μl 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 μl 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 μl 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 μl of 1N H_2SO_4 to each well and read the plate at 450 nm.

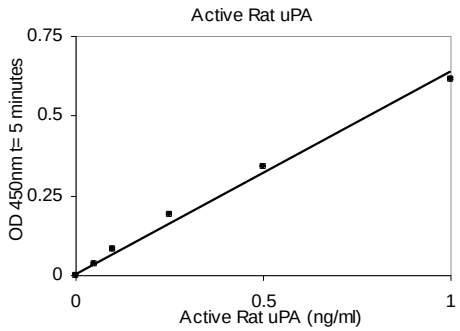
Scheme I:

	1	2	3	4	5	6	7	8	9	10	11	12
A	S_9	S_8	S_7	S_6	S_5	S_4	S_3	S_2	S_1	B_0	U_1	U_2
B	S_9	S_8	S_7	S_6	S_5	S_4	S_3	S_2	S_1	B_0	U_1	U_2
C	U_3	U_4	U_5	U_6	U_7	U_8	U_9	U_{10}	U_{11}	U_{12}	U_{13}	U_{14}
D	U_3	U_4	U_5	U_6	U_7	U_8	U_9	U_{10}	U_{11}	U_{12}	U_{13}	U_{14}
E	U_{15}	U_{16}	U_{17}	U_{18}	U_{19}	U_{20}	U_{21}	U_{22}	U_{23}	U_{24}	U_{25}	U_{26}
F	U_{15}	U_{16}	U_{17}	U_{18}	U_{19}	U_{20}	U_{21}	U_{22}	U_{23}	U_{24}	U_{25}	U_{26}
G	U_{27}	U_{28}	U_{29}	U_{30}	U_{31}	U_{32}	U_{33}	U_{34}	U_{35}	U_{36}	U_{37}	U_{38}
H	U_{27}	U_{28}	U_{29}	U_{30}	U_{31}	U_{32}	U_{33}	U_{34}	U_{35}	U_{36}	U_{37}	U_{38}

CALCULATIONS

1. Plot the A_{450} 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



EXPECTED VALUES

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).

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

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