

Rat Urokinase Plasminogen Activator (uPA) Total Antigen ELISA

For Research Use Only

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).

PRINCIPLES OF PROCEDURE

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 anti-rat 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.

MATERIALS PROVIDED

Component	Contents	Quantity	Storage	Cat. No.
Coated Plate	Capture antibody coated 96-well plate	1 plate	4°C	UP42a
Standard	Rat uPA activity standard (lyophilized)	1 vial	4°C	UP42b
Primary Antibody	Rabbit anti-rat uPA antibody (lyophilized)	1 vial	4°C	UP42c
Wash Buffer	10x solution for washing plate	50 mL	4°C	UP42d
Substrate	TMB substrate	10 mL	4°C	UP42e
Secondary Antibody	Anti-rabbit HRP conjugated antibody	1 vial	4°C	UP42f

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 H₂SO₄
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 Standards and Primary Antibody may be stored at -70°C for later use. **DO NOT** freeze/thaw the Standards 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. 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. 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.**

Table 1: Preparation of Standard Curve

Standard	uPA Concentration (ng/mL)	Blocking Buffer (µL)	Transfer Volume (µL)	Transfer Source	Final Volume (µL)
S ₁₀	10	900	100	Stock	500
S ₉	5	500	500	S ₁₀	600
S ₈	2	600	400	S ₉	500
S ₇	1	500	500	S ₈	500
S ₆	0.5	500	500	S ₇	500
S ₅	0.25	500	500	S ₆	600
S ₄	0.1	600	400	S ₅	500
S ₃	0.05	500	500	S ₄	500
S ₂	0.025	500	500	S ₃	600
S ₁	0.01	600	400	S ₂	1,000
B ₀	0	500	---	---	500

ASSAY PROCEDURE

1. Add 100 μ L of the Standards and unknowns to the wells in duplicate. See Scheme I for a sample plate layout. 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 μ 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 μ L of the Primary Antibody to each well. Shake the plate at 300 rpm for 30 minutes at RT.
4. Wash the plate three times as in step 2.
5. Add 100 μ L of the Secondary 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 μ L of TMB Substrate to each well. Shake the plate at 300 rpm for 2-10 minutes at RT.
8. Stop the reaction by adding 50 μ L of 1 N H₂SO₄ to each well and read the plate at 450 nm.

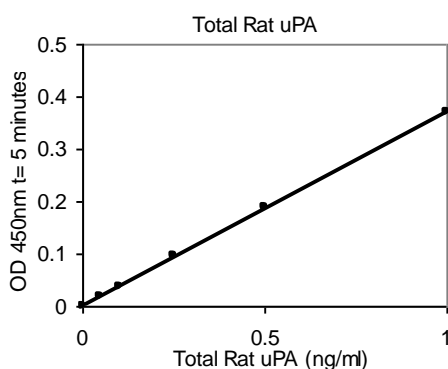
Scheme I:

	1	2	3	4	5	6	7	8	9	10	11	12
A	S ₁₀	S ₉	S ₈	S ₇	S ₆	S ₅	S ₄	S ₃	S ₂	S ₁	B ₀	U ₁
B	S ₁₀	S ₉	S ₈	S ₇	S ₆	S ₅	S ₄	S ₃	S ₂	S ₁	B ₀	U ₁
C	U ₂	U ₃	U ₄	U ₅	U ₆	U ₇	U ₈	U ₉	U ₁₀	U ₁₁	U ₁₂	U ₁₃
D	U ₂	U ₃	U ₄	U ₅	U ₆	U ₇	U ₈	U ₉	U ₁₀	U ₁₁	U ₁₂	U ₁₃
E	U ₁₄	U ₁₅	U ₁₆	U ₁₇	U ₁₈	U ₁₉	U ₂₀	U ₂₁	U ₂₂	U ₂₃	U ₂₄	U ₂₅
F	U ₁₄	U ₁₅	U ₁₆	U ₁₇	U ₁₈	U ₁₉	U ₂₀	U ₂₁	U ₂₂	U ₂₃	U ₂₄	U ₂₅
G	U ₂₆	U ₂₇	U ₂₈	U ₂₉	U ₃₀	U ₃₁	U ₃₂	U ₃₃	U ₃₄	U ₃₅	U ₃₆	U ₃₇
H	U ₂₆	U ₂₇	U ₂₈	U ₂₉	U ₃₀	U ₃₁	U ₃₂	U ₃₃	U ₃₄	U ₃₅	U ₃₆	U ₃₇

CALCULATIONS

1. Plot the A₄₅₀ 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 from 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

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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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Oxford Biomedical Research, Inc.
P.O. Box 522
Oxford, MI 48371 U.S.A.

Orders: 800-692-4633
Technical Service: 248-852-8815
Fax: 248-852-4466
E-mail: info@oxfordbiomed.com

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