Catalog # RUPAKT-TOT

Rat uPA Total Antigen Assay

Strip well format. Reagents for up to 96 tests.

For Research Use Only.

INTENDED USE

Rat uPA total antigen assay is intended for the quantitative determination of total plasminogen activator antigen in rat plasma.

BACKGROUND

Urokinase plasminogen activator 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].

ASSAY PRINCIPLE

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 450nm. A standard calibration curve is prepared along with the samples to be measured using dilutions of uPA.

REAGENTS PROVIDED

- **Coated plate:**
1-96 well immulon plate (8X12 removable wells) coated, blocked, and dried with capture antibody
- **10X Wash Buffer:**
1 bottle of 50ml wash; bring to 1X using DI water
- **Rat uPA activity standard:**
1 vial lyophilized standard
- **Anti-rat uPA primary antibody:**
1 vial lyophilized polyclonal anti-rat uPA antibody
- **Anti-rabbit horseradish peroxidase conjugate secondary antibody:**
1 vial HRP labeled antibody
- **TMB substrate solution:** 10 ml

STORAGE AND STABILITY

All other reagents must be stored at 4°C. Store unopened plate and any unused microtiter strips in the pouch with desiccant. Kit should be used no later than the expiration date. **DO NOT** freeze/thaw the primary antibody more than once.

REAGENTS AND EQUIPMENT REQUIRED

- 1-channel pipettes covering 20-200 µl, 500-5000 µl and 200-1000µl
- 12-channel pipette for 30-300µl
- Paper towels or kimwipes
- 1.5ml micro centrifuge tubes
- 1N H_2SO_4
- DI water
- Magnetic stirrer and stir-bars
- Plastic containers with lids

•TBS buffer
•Blocking buffer
•Microtiter plate spectrophotometer operable at 450nm
•Microtiter plate shaker with uniform horizontally circular movement up to 300rpm

WARNINGS

Warning – Avoid skin and eye contact when using TMB One substrate solution since it may be irritating to eyes, skin, and respiratory system. Wear safety goggles and gloves.

PRECAUTIONS

- **DO NOT** mix any reagents or components of this kit with any reagents or components of any other kit. This kit is designed to work properly as provided.
- **DO NOT** pipette reagents by mouth.
- Always pour substrate out of the bottle into a clean test tube. **DO NOT** pipette out of the bottle as you could contaminate the substrate.
- Keep plate covered except when adding reagents, washing, or reading.
- **DO NOT** smoke, drink, or eat in areas where specimens or reagents are being handled.

PREPARATION OF REAGENTS

•**TBS buffer:** 0.10M TRIS, 0.15M NaCl, pH 7.4
•**Blocking buffer (BSA):** 3% BSA in TBS buffer

SPECIMEN COLLECTION

Collect 9 volumes of blood in 1 volume of 0.1M trisodium citrate or acidified citrate, preferably using Stabilyte ™ evacuated vials (Biopool, cat# 102080). Immediately after collection of blood, samples must be centrifuged at 3000Xg for 15 minutes. The plasma must be transferred to a clean plastic tube and must be stored on ice prior to anaylsis. 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 0.01-10 ng/ml range. Samples giving uPA levels above 10ng/ml should be diluted in plasma devoid of uPA.

ASSAY PROCEDURE

Perform assay at room temperature. Vigorously shake plate (300rpm) at each step of the assay.

Preparation of Standard:
Dilution table for preparation of rat uPA standards:

Rat uPA: Reconstitute vial with 1ml blocking buffer for a final concentration of **100ng/ml**

uPA concentration (ng/ml)	Dilutions
10	900µl (BSA) + 100µl (100ng/ml)
5	500µl (BSA) + 500µl (10ng/ml)
2	600µl (BSA) + 400µl (5ng/ml)
1	500µl (BSA) + 500µl (2ng/ml)
0.5	500µl (BSA) + 500µl (1ng/ml)
0.25	500µl (BSA) + 500µl (0.5ng/ml)
0.1	600µl (BSA) + 400µl (0.25ng/ml)
0.05	500µl (BSA) + 500µl (0.1ng/ml)
0.025	500µl (BSA) + 500µl (0.05ng/ml)
0.01	600µl (BSA) + 400µl (0.05ng/ml)
0	500µl (BSA) Zero point to determine background

NOTE: DILUTIONS FOR THE STANDARD CURVE AND ZERO STANDARD MUST BE MADE AND APPLIED TO THE PLATE IMMEDIATELY.

Standard and Unknown Addition:
Remove microtiter plate from bag. Add 100µl standard in duplicate and unknown to wells. Record positions of standards and unknowns. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Primary Antibody Addition:
Reconstitute primary antibody by adding 10ml blocking buffer to vial. Agitate gently to completely dissolve contents. Add 100µl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

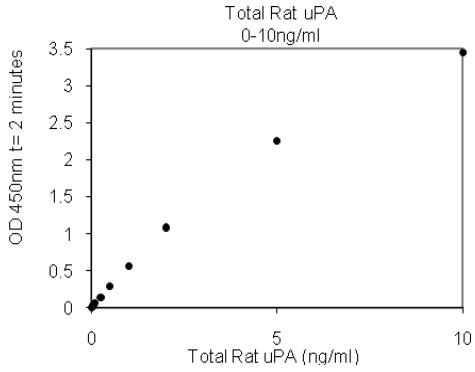
Secondary Antibody Addition:
Dilute 1µl into 15ml of 3% BSA blocking buffer and add 100µl to all wells. Wash wells three times with 300µl wash buffer. Shake plate at 300rpm for 30 minutes. Remove excess wash by gently tapping plate on paper towel or kimwipe.

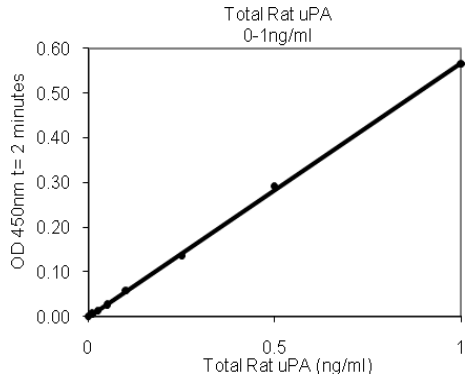
Substrate Incubation:
Add 100µl TMB substrate to all wells and shake plate for 2-10 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50µl of 1N H_2SO_4 stop solution to all wells when samples are visually in the same range as the standards. Add stop solution to wells in the same order as substrate upon which color will change from blue to yellow. Mix thoroughly and read final absorbance values at 450nm. For best results read plate immediately.

Measurement:
Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm. Subtract zero point from all standards and unknowns to determine corrected absorbance (A_{450}).

Assay Calibration:
Plot A_{450} against the amount of uPA in the standards. Fit a straight line through the points using a linear fit procedure. The uPA activity in the unknowns can be determined by from this curve.

A typical standard curve.
(EXAMPLE ONLY, DO NOT USE)





EXPECTED VALUES

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

♦ Venous Thrombosis: Low levels of uPA is associated with clot formation [2].

♦ Inflammatory Disease: Low levels of uPA may aggravate this condition [4].

QUALITY CONTROL

The performance of each assay can be controlled using a positive quality control sample. An abnormally high uPA sample can be prepared by freezing aliquots of plasma known to contain a high level of uPA.

DISCLAIMER

This information is believed to be correct but does not claim to be all-inclusive and shall be used only as a guide. The supplier of this kit shall not be held liable for any damage resulting from handling or from contact with the above product.

REFERENCE

1. Declerck PJ, *et al*.: Immunoassay of murine t-PA, u-PA, and PAI-1 using monoclonal antibodies raised in gene-inactivated mice. Thromb Haemostas., Nov**74(5)**: 1305-9, 1995.
2. Singh I, *et al.*: Failure of thrombus to resolve in urokinase-type plasminogen activator gene-knockout mice: rescue by normal bone marrow-derived cells. Circulation, **107(6)**, 869-875, 2003.
3. Kjøller Lars: The Urokinase Plasminogen Activator Receptor in the Regulation of the Actim Cytoskeleton and Cell Motility. Biol. Chem., **383**: 5-19, 2002.
4. Yang YH, *et al*.: Tissue-type plasminogen activator deficiency exacerbates arthritis. J. Immunol., **167(2)**, 1047-52, 2001.

Example of 96 Well Plate Layout
Standards:22 wells
Samples:74 wells

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0.01ng/ml	0.025ng/ml	0.05ng/ml	0.1ng/ml	0.25ng/ml	0.5ng/ml	1ng/ml	2ng/ml	5ng/ml		
B	0	0.01ng/ml	0.025ng/ml	0.05ng/ml	0.1ng/ml	0.25ng/ml	0.5ng/ml	1ng/ml	2ng/ml	5ng/ml		
C												
D												
E												
F												
G												
H												