Total Mouse uPA Assay

For Research Use Only

INTRODUCTION

Urokinase-type Plasminogen Activator (uPA) is a glycosylated serine protease that is synthesized in endothelial and kidney epithelial cells. There are two forms of uPA, the low molecular weight (LMW ~31 kDa) and high molecular weight (HMW ~55 kDa). The HMW form undergoes autoproteolysis resulting in the LMW plus an 18.5 kDa amino terminal fragment (ATF). It is this ATF that has been shown to inhibit proliferation and invasion of cancer cells by binding to uPA receptors(1).

PRINCIPLES OF PROCEDURE

This is an ELISA (Enzyme-Linked Immunosorbent Assay) for the quantitative analysis of total uPA levels in biological fluid. This test kit operates on the basis of sandwich ELISA where active, latent and complexed uPA enzyme complexes with PAI-1 and is quantitated with the use of an HRP labeled secondary antibody.

First the biotinylated PAI-1 binds to the avidin coated wells. Next, active, latent or complexed uPA present in the standard or unknown samples, complexes with PAI-1. A primary antibody specific for the various uPA forms is then added to each well followed by the HRP conjugated secondary antibody. The bound conjugated secondary antibody is detected by the addition of substrate, which generates an optimal color after 10 minutes. Quantitative test results may be obtained by the measure and comparison of the sample and standard absorbance readings when read with a microplate reader at 450 nm.

MATERIALS PROVIDED

Component	Contents	Quantity	Storage	Cat. No.
Coated Plate	Capture antibody coated 96-well plate	1 plate	4°C	PL95a
Standard	Mouse uPA activity standard (lyophilized)	1 vial	4°C	PL95b
Primary Antibody	Anti-mouse uPA antibody (lyophilized)	1 vial	4°C	PL95c
10x Wash Buffer	10x solution for washing plate	50 mL	4°C	PL95d
TMB Substrate	TMB Substrate	10 mL	4°C	PL95e
Secondary Antibody	Anti-rabbit HRP conjugated antibody	1 vial	4°C	PL95f

MATERIALS NEEDED BUT NOT PROVIDED

1. 1 N H_2SO_4
2. TBS Buffer (see Reagent Preparation)
3. Blocking Buffer (see Reagent Preparation)
4. DI Water
5. Microplate reader with 450 nm filter
6. Microplate shaker with uniform horizontal circular movement up to 300 rpm
7. Precision pipettes that range from 10 µL-1000 µL and disposable tips

STORAGE

1. Store the kit and all of its components at 4°C before use.

2. If not using the entire plate at once, prepare only the appropriate amount of Primary Antibody, Secondary Antibody and uPA Standard. The remaining stock solutions of the Primary Antibody and the uPA Standard should be aliquoted, frozen and stored at –70°C. **DO NOT** freeze/thaw the Primary Antibody or Standard more than once. All other components should remain refrigerated. Store unused portions of the microplate in a pouch with a desiccant at 4°C.

WARNINGS AND PRECAUTIONS

1. 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.
2. Exercise universal precautions during the performance or handling of this kit or any component contained therein.

PROCEDURAL NOTES

1. This assay should be run at room temperature.
2. Use aseptic technique when opening and dispensing reagents.
3. To minimize error due to handling, wipe the exterior bottom of the microplate wells with a lint-free paper towel.

SAMPLE COLLECTION, STORAGE, AND PREPARATION

Samples should be collected using 0.01 M trisodium citrate, acidified citrate or Stabilyte^{tm} (Biopool, cat# 102080) collection media. Collection should be in accordance with the collection vial manufacturer's instructions or in a 1:10 ratio of collection media to blood.

Immediately upon collection of blood, the samples should be centrifuged at 3000 x g for 15 minutes. This should ensure the removal of platelets as they can release PAI-1 that, in turn, complexes with uPA. The plasma can be transferred to a clean plastic tube and stored frozen for up to one month. Samples are stable for approximately 24 hours when stored at 4°C with the Sabilyte^{tm} media or one month if stored at -20°C.

Note: Detergents such as Triton X cause interference with the assay. If using detergent extracted samples, it is necessary to dialyze the samples overnight to remove the detergent.

REAGENT PREPARATION

The following solutions should be prepared fresh before starting the assay.

1. **TBS Buffer:** 0.1 M TRIS, 0.15 M NaCl, pH 7.4
2. **3% BSA Blocking Buffer:** 3% BSA in TBS Buffer.
3. **10x Wash Buffer:** Dilute the 50 mL of concentrate to 1x with 450 mL of DI water prior to use.
4. **Standard:** Reconstitute with 3% BSA Blocking Buffer as directed on the vial and vortex gently to mix. Prepare according to included Standard Dilution Table 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.

ASSAY PROCEDURE

1. Add 100 µl of the Standards and unknowns to the wells in duplicate. Dilutions of the unknown may be made in 3% BSA Blocking Buffer. For a suggested plate layout, see Scheme I below. Shake the plate at 300 rpm for 30 minutes at 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. Remove the contents of each well by inversion of plate into an appropriate disposal device.
f. Repeat procedure two more times, then proceed to step "g".
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 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 µl of the Secondary Antibody to each well. Shake the plate at 300rpm for 30 minutes at RT.
6. Wash the plate three times as in step 2.
7. Add 100 µl of TMB Substrate to each well. Shake the plate at 300 rpm for 10 minutes at RT.
8. Stop the reaction by adding 50 µl of 1N H_2SO_4 to each well and read the plate at 450 nm.

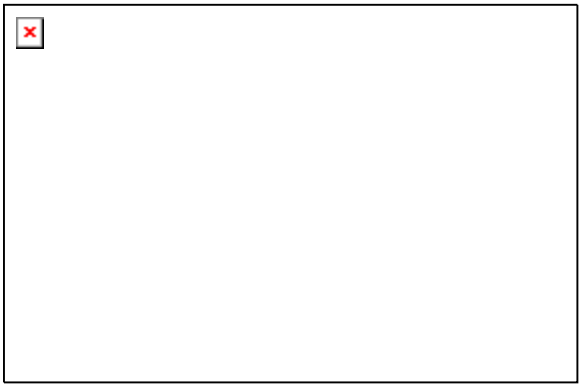
Scheme I:

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	S_0	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	U_1	U_2	U_3
B	S_0	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	U_1	U_2	U_3
C	U_4	U_5	U_6	U_7	U_8	U_9	U_{10}	U_{11}	U_{12}	U_{13}	U_{14}	U_{15}
D	U_4	U_5	U_6	U_7	U_8	U_9	U_{10}	U_{11}	U_{12}	U_{13}	U_{14}	U_{15}
E	U_{16}	U_{17}	U_{18}	U_{19}	U_{20}	U_{21}	U_{22}	U_{23}	U_{24}	U_{25}	U_{26}	U_{27}
F	U_{16}	U_{17}	U_{18}	U_{19}	U_{20}	U_{21}	U_{22}	U_{23}	U_{24}	U_{25}	U_{26}	U_{27}
G	U_{28}	U_{29}	U_{30}	U_{31}	U_{32}	U_{33}	U_{34}	U_{35}	U_{36}	U_{37}	U_{38}	BLK
H	U_{28}	U_{29}	U_{30}	U_{31}	U_{32}	U_{33}	U_{34}	U_{35}	U_{36}	U_{37}	U_{38}	BLK

CALCULATIONS

1. Average the O.D. values for each pair of duplicate wells.
2. Plot the A_{450} against the concentration of uPA in the standards.
3. Fit a straight line through the points using a linear fit procedure.
4. Calculate the uPA concentrations in the unknowns using the standard curve.

Typical Standard Curve:



PERFORMANCE CHARACTERISTICS

Assay Range: 0.125-10 ng/mL

Samples with uPA levels higher than 10 ng/mL should be diluted in similar media devoid of active uPA or 3% BSA Blocking Buffer.

REFERENCES

1. Luparello C et al. (1996) Eu J Cancer A: 702-707

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