ab198512 –

uPA Total (URK) Mouse ELISA Kit

Instructions for Use

For the quantitative measurement of total plasminogen activator antigen in mouse plasma.

This product is for research use only and is not intended for diagnostic use.

Version 1 Last Updated 15 January 2019

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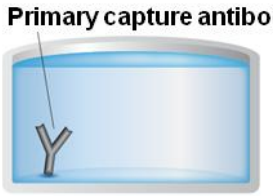
1. BACKGROUND

Abcam's uPA Total (URK) *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of total plasminogen activator antigen in mouse plasma.

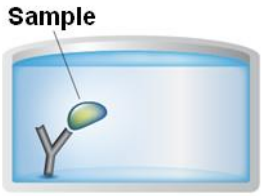
Mouse uPA will bind to the capture antibody coated on the microtiter plate. Free and complexed enzyme will react with the capture antibody on the plate. After appropriate washing steps, polyclonal anti-murine uPA primary antibody binds to the captured enzyme. Excess antibody is washed away and bound 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.

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.

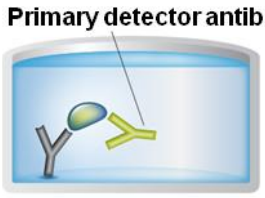
2. ASSAY SUMMARY



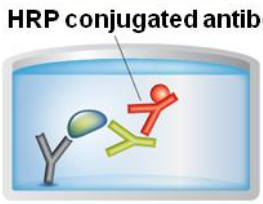
Prepare all reagents, samples, and controls as instructed. Plate is supplied pre-coated with capture antibody.



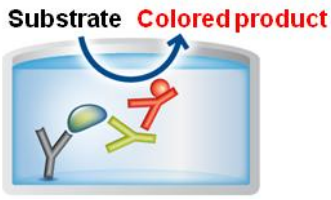
Add sample to appropriate wells. Incubate at room temperature.



Add primary detection antibody. Incubate at room temperature.



Aspirate and wash each well. Add HRP conjugated secondary antibody, which binds the primary antibody. Incubate at room temperature.



Aspirate and wash each well. Add developing solution until color develops and then add the stop solution. Immediately begin recording the color development.

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store all kit components at +2-8°C upon arrival.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Reagent Preparation section.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
uPA antibody pre-coated 96 well microplate (12 x 8 well strips)	1X 96 tests	+2-8°C
10X Wash Buffer	1X 50 mL	+2-8°C
Mouse uPA standard	1 vial	+2-8°C
Anti-mouse uPA primary antibody	1 vial	+2-8°C
Anti-rabbit horseradish peroxidase secondary antibody	1 vial	+2-8°C
TMB substrate solution	1X 10 mL	+2-8°C

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microtiter plate shaker capable of 300 rpm uniform horizontally circular movement
- Manifold dispenser/aspirator or automated microplate washer
- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes and Pipette tips
- Deionized or distilled water
- Polypropylene tubes for dilution of standard
- Paper towels or laboratory wipes
- 1N H_2SO_4 or 1N HCl
- Bovine Serum Albumin Fraction V (BSA)
- Tris(hydroxymethyl)aminomethane (Tris)
- Sodium Chloride (NaCl)

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted

8. TECHNICAL HINTS

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Always pour peroxidase substrate out of the bottle into a clean test tube. Do not pipette out of the bottle as contamination could result.
- Complete removal of all solutions and buffers during wash steps.
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**

9. REAGENT PREPARATION

Equilibrate all reagents and samples to room temperature (18-25°C) prior to use.

9.1. **1X Wash Buffer**

Dilute 50 mL of 10X Wash Buffer with 450 mL of deionized water.

9.2. **1X TBS buffer**

0.1M Tris, 0.15M NaCl, pH 7.4

9.3. **1X Blocking Buffer**

3% BSA (w/v) in TBS.

9.4. **uPA Primary Antibody**

Reconstitute uPA Primary Antibody by adding 10 mL of 1X Blocking Buffer directly to the vial and agitate gently to completely dissolve contents**.** Reconstituted uPA Standards and uPA Primary Antibody may be stored at -80°C for later use.

10. STANDARD PREPARATIONS

Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use.

10.1 Reconstitute Mouse uPA Standard by adding 1ml of 1X Blocking Buffer directly to the vial and agitate gently to completely dissolve contents. This will result in a 100 ng/mL **Standard #1**.

10.2 Label eleven further tubes # 2-12.

10.3 Prepare **Standard #2,** in tube #2 by adding 100 µL (100 ng/mL) **Standard #1** to 900 µL 1X Blocking Buffer and mix gently and thoroughly.

10.4 Using the table below as a guide, prepare further serial dilutions using 1X Blocking Buffer as diluent.

10.5 1X Blocking Buffer serves as the zero standard, 0 ng/mL (Tube #12).

Standard Dilution Preparation Table

Standard #	Sample to Dilute	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (ng/mL)	Final Conc. (ng/mL)
1	See section 10.1				100
2	Standard 1	100	900	100	10
3	Standard #2	500	500	10	5
4	Standard #3	400	600	5	2
5	Standard #4	500	500	2	1
6	Standard #5	500	500	1	0.5
7	Standard #6	500	500	0.5	0.25
8	Standard #7	400	600	0.25	0.1
9	Standard #8	500	500	0.1	0.05
10	Standard #9	500	500	0.05	0.025
11 (Blank)	-	-	500	-	0

11. SAMPLE COLLECTION AND STORAGE

11.1 Collect plasma using EDTA or citrate as an anticoagulant.

11.2 Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection.

11.3 Assay immediately or aliquot and store at ≤ -20°C. Avoid repeated freeze-thaw cycles.

12. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents
- Unused well strips should be returned to the plate packet and stored at +2-8°C
- For each assay performed, a minimum of 2 wells must be used as blanks, omitting primary antibody from well additions
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates)
- Well effects have not been observed with this assay. Contents of each well can be recorded on the template sheet included in the Resources section

13. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**
- **Return any unused microplate strips to the plate pouch with desiccant. Reconstituted standards and primary may be stored at -80°C for later use. Do not freeze-thaw the standard and primary antibody more than once. Store all other unused kit components at +2-8°C.**

13.1 Prepare all reagents, working standards, and samples as directed in the previous sections.

13.2 Remove microtiter plate from bag and add 100 µL uPA Standards (in duplicate) and Samples to wells.

13.3 Carefully record position of standards and Samples.

13.4 Shake plate at 300 rpm for 30 minutes.

13.5 Wash wells three times with 300 µL 1X Wash Buffer. Remove excess 1X Wash Buffer by gently tapping plate on paper towel.

NOTE: The assay measures uPA antigen in the 0.025-10 ng/mL range. If the unknown is thought to have high uPA levels, dilutions may be in plasma devoid of uPA or in Blocking Buffer.

13.6 Add 100 µL reconstituted uPA Primary Antibody to all wells.

13.7 Shake plate at 300 rpm for 30 minutes.

13.8 Wash wells three times with 300 µL 1X Wash Buffer. Remove excess 1X Wash Buffer by gently tapping plate on paper towel.

13.9 Dilute 1 µL of HRP conjugated Secondary Antibody in 10 mL of 1X Blocking Buffer and add 100 µL to all wells.

13.10 Shake plate at 300 rpm for 30 minutes.

13.11 Wash wells three times with 300 µL 1X Wash Buffer. Remove excess 1X Wash Buffer by gently tapping plate on paper towel.

13.12 Add 100 µL TMB substrate to all wells and shake plate for 2-10 minutes. Substrate will change from colorless to different strengths of blue.

13.13 Quench reaction by adding 50 µL of 1N H_2SO_4 or HCl 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 by gently shaking the plate.

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

14. CALCULATIONS

Plot A_{450} against the amount of uPA in the standards. Fit a straight line through the linear points of the standard curve using a linear fit procedure if unknowns appear on the linear portion of the standard curve. Alternatively, create a standard curve by analysing the data using a software program capable of generating a four parameter logistic (4PL) curve fit. The amount of uPA in the Samples can be determined from this curve. If samples have been diluted, the calculated concentration must be multiplied by the dilution factor.

The concentration level of uPA antigen in murine urine has been reported to be 1.8+/-1.9 µg/mL. In house testing of pooled normal mouse plasma in citrate indicates uPA levels vary by mouse strain.

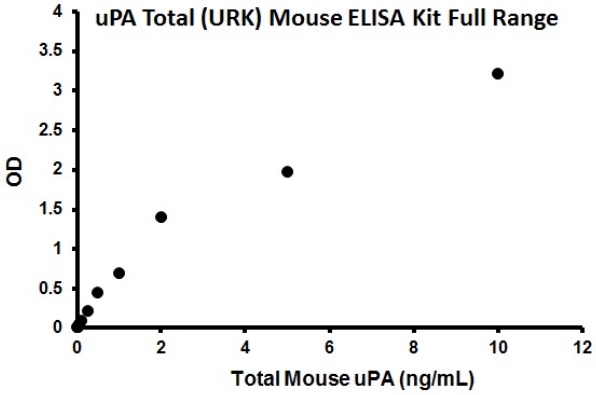
Strain	Total uPA
NSA/CF-1	2.6 ng/mL
C57BL6	1.4 ng/mL
CD-1	0.3 ng/mL

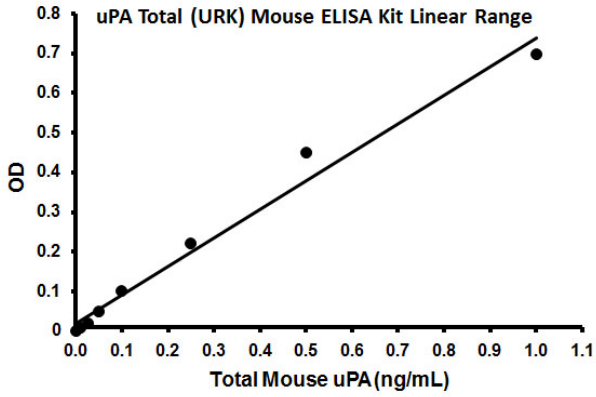
Abnormalities in uPA levels have been reported in the following conditions:

- Venous Thrombosis: Low levels of uPA is associated with clot formation.
- Inflammatory Disease: Low levels of uPA may aggravate this condition.

15. TYPICAL DATA

TYPICAL STANDARD CURVE – Graphs provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed





16. TYPICAL SAMPLE VALUES

INTRA-ASSAY PRECISION

Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Sample Type	1	2	3
n	20	20	20
Mean (ng/mL)	0.06	0.30	3.01
Standard Deviation	0.005	0.019	0.112
CV (%)	8.12	6.18	3.73

INTER-ASSAY PRECISION

Three samples of known concentration were tested in ten independent assays to assess inter-assay precision.

Sample Type	1	2	3
n	10	10	10
Mean (ng/mL)	0.09	0.25	2.66
Standard Deviation	0.008	0.019	0.154
CV (%)	8.52	7.47	5.79

SENSITIVITY

The minimum detectable dose (MDD) was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates (range OD_{450}: 0.063-0.08) and calculating the corresponding concentration. The MDD was 0.015 ng/mL.

RECOVERY

The recovery of antigen spiked to levels throughout the range of the assay in Blocking Buffer was evaluated.

Sample	1	2	3	4
n	4	4	4	4
Mean (ng/mL)	0.03	0.19	1.49	3.20
Average % Recovery	108	93	99	91
Range %	92 - 115	88 - 99	94 - 103	83 - 107

LINEARITY OF DILUTION

To assess the linearity of the assay, pooled mouse plasma samples containing high concentrations of antigen were serially diluted to produce samples with values within the dynamic range of the assay.

Sample	1:2	1:4	1:8	1:16
n	4	4	4	4
Average % of Expected	101	96	102	105
Range %	99 - 104	92 - 103	99 - 104	103 - 106

17. ASSAY SPECIFICITY

This assay recognizes natural and recombinant total mouse uPA. Pooled normal plasma from rat and pig was assayed and significant cross-reactivity was observed. Pooled normal plasma from sheep was assayed and no significant cross-reactivity was observed. Pooled normal plasma from rabbit resulted in significant color development.

18. TROUBLESHOOTING

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting Improper standards dilution	Check pipettes Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions
	Contaminated Wash Buffer	Prepare fresh Wash Buffer
Low sensitivity	Improper storage of the ELISA kit	Store the reconstituted protein at -80°C, all other assay components 2-8°C. Keep substrate solution protected from light.

19. NOTES

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