ab108916 Urokinase type plasminogen activator Human Chromogenic Activity Assay Kit (Direct Assay)

Instructions for Use

For the quantitative measurement of Human Urokinase type plasminogen activator concentrations in plasma, serum and cell culture supernatants

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

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Table of Contents

1. Introduction

Urokinase type plasminogen activator (uPA) is a highly restricted serine protease that converts the zymogen plasminogen to active plasmin, a broad-spectrum serine proteinase capable of degrading most of the major protein components of the extracellular matrix. Binding of uPA to its receptor and subsequent uPA mediated pericellular proteolysis are involved in many process including cell migration and tissue remodeling in angiogeenesis, atherogenesis, tumor cell metastasis, and ovulation. A high level of uPA is a poor prognostic marker for aggressive breast cancer, aggressive prostate cancer, bladder cancer and gastric cancer.

ab108916 uPA Human Chromogenic Activity Assay Kit is developed to determine uPA activity in Human plasma, serum and cell culture supernatants. The amidolytic activity of uPA is quantitated using a highly specific uPA substrate releasing a yellow para-nitroaniline (pNA) chromophore. The change in absorbance of the pNA at 405 nm is directly proportional to the uPA enzymatic activity.

2. Assay Summary

Prepare all reagents, samples and standards as instructed.

Add 50 µl of Assay Diluent per well.

Add 30 µl of Standard or Sample per well.

Add 30 µl of uPA Substrate per well.

Read the absorbance at 405 nm for a zero minute background reading.

Cover and incubate at 37°C.

Read every 3 minutes for 15 minutes (High Activity).

Read every 30 minutes for 2 hours (Low Activity).

3. Kit Contents

The activity kit contains sufficient reagents to perform 96 tests using microplate method.

- Microplate: A 96-well polystyrene microplate (12 strips of 8 wells).
- Sealing Tapes: Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.
- Assay Diluent (1x): Buffered protein base (30 ml).
- uPA Standard: 1 vial Human high molecular weight uPA
- uPA Substrate: Lyophilised Substrate (3 vials).

4. Storage and Handling

Store components of the kit at 2-8°C or -20°C upon arrival up to the expiration date. Store Human uPA Standard and uPA Substrate at -20°C. Store Microplate and Assay Diluent at 2-8°C. Opened unused microplate wells may be returned to the foil pouch with the desiccant packs. Reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator. Diluent (1x) may be stored for up to 1 month at 2-8°C.

All Human source materials have been tested and found to be negative to HbsAg, HIV-1 and HCV by FDA approved methods.

5. Additional Materials Required

- Microplate reader capable of measuring absorbance at 405nm.
- Precision pipettes to deliver 1 µl to 1 ml volumes.
- Distilled or deionized reagent grade water.
- Incubator (37°C).

6. Preparation of Reagents

Sample Collection:

1. **Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 2,000 x *g* for 10 minutes and assay. The sample is suggested for use at 1x or within the range of 2x – 5x into Assay Diluent; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as an anticoagulant).
2. **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x g for

10 minutes, remove serum, and assay. The sample is suggested for use at 1x or within the range of 2x – 5x into Assay Diluent; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

3. **Cell culture supernatants:** Centrifuge cell culture media at 1500 rpm for 10 minutes at 4°C to remove debris and collect supernatant. If necessary, dilute samples into Assay Diluent; user should determine optimal dilution factor depending on application needs. Samples can be stored at -80°C. Avoid repeated freeze-thaw cycles.

Reagent Preparation:

Freshly dilute all reagents and bring all reagents to room temperature before use.

1. uPA Substrate: Add 1.1 ml of reagent grade water to produce a 1x stock solution. Allow the vial to sit for 10 minutes with gentle agitation prior to use. Any remaining stock solution should be stored at -20°C and used within 30 days. Avoid repeated freeze-thaw cycles.
2. **Standard Curve:** Reconstitute the uPA Standard with appropriate amount of Diluent to generate a stock solution of 25 IU/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions.

For high level of uPA activity samples, prepare triplicate standard points by serially diluting the standard solution (25 IU/ml) two-fold with equal volume of Diluent to produce 12.5, 6.25, 3.125, 1.563 and 0.781 IU/ml. Any remaining solution should be frozen at -20^0C.

Standard curve for high level of uPA activity samples:

Standard Point	**Dilution**	**[H. uPA] (IU/ml)**
P1	Standard (25 IU/ml) + 1 part Diluent	25
P2	1 part P1 + 1 part Diluent	12.50
P3	1 part P2 + 1 part Diluent	6.25
P4	1 part P3 + 1 part Diluent	3.125
P5	1 part P4 + 1 part Diluent	1.563
P6	Diluent	0.781
P7	Diluent	0.000

For low level of uPA activity samples, dilute the stock solution (25 IU/ml) 1:8 with Assay Diluent to yield a solution of 3.125 IU/ml. Prepare duplicate or triplicate standard points by

serially diluting the standard solution (3.125 IU/ml) two-fold with equal volume of Assay Diluent to produce 1.563, 0.781, 0.391, 0.195 and 0.098 IU/ml. Assay Diluent serves as the zero standard (0 IU/ml). Any remaining solution should be frozen at -20^{0}C.

Standard curve for low level of uPA activity samples:

Standard Point	**Dilution**	**[H. uPA] (IU/ml)**
P1	Standard (25 IU/ml) + 7 part Diluent	3.125
P2	1 part P1 + 1 part Diluent	1.563
P3	1 part P2 + 1 part Diluent	0.781
P4	1 part P3 + 1 part Diluent	0.391
P5	1 part P4 + 1 part Diluent	0.195
P6	1 part P5 + 1 part Diluent	0.098
P7	Diluent	0.000

7. Assay Method

1. Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at 37°C in a humid incubator.
2. Remove excess microplate strips from the plate frame.
3. Add 50 µl of the Assay Diluent to each well of the 96-well plate. Gently tap plate to thoroughly coat the wells.
4. Add 30 µl of Human uPA Standard or sample to each well.
5. Add 30 µl of uPA Substrate to each well. Gently tap plate to ensure thorough mixing. Break any bubbles that may have formed. Read the absorbance at 405 nm for a zero minute background reading. Cover wells with a sealing tape and incubate at 37°C in a humid incubator to avoid evaporation. Incubate microplate at 37°C after each reading.

Assay Diluent	50 µl
uPA or Samples	30 µl
uPA Substrate	30 µl

High uPA activity Samples: *37*°C*,* read the absorbances at 405 nm every 3 minutes for 15 minutes

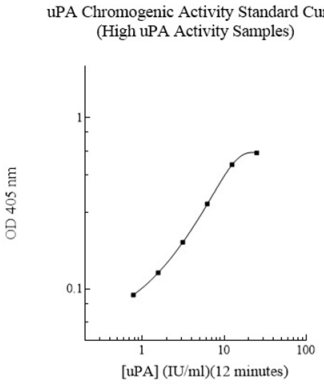
Low uPA Activity samples: *37*°C*,* read the absorbances at 405 nm every 30 minutes up to 2 hours

8. Data Analysis

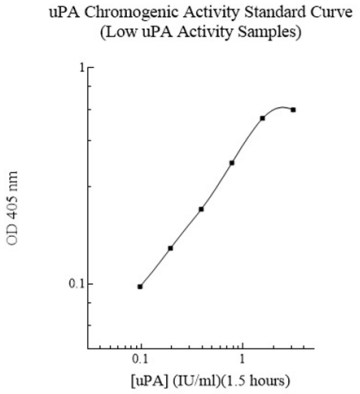
Calculate the mean value of the triplicate readings for each standard and sample. To generate a Standard Curve, from the initial reaction time, plot the graph using the standard concentrations on the x-axis and the corresponding mean 405 nm absorbance or change in absorbance per minute on the y-axis after. The best-fit line can be determined by regression analysis of the linear portion of the curve. Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

A. Typical Data

The curves are provided for illustration only. A standard curve should be generated each time the assay is performed. For High uPA Activity Samples see graph below.



For Low uPA Activity:



9. Specificity

The minimum detectable dose of human uPA is approximately 0.062 IU/ml.

10. Troubleshooting

Problem	Cause	Solution
Poor standard curve	Improper standard dilution	Confirm dilutions made correctly
	Standard improperly reconstituted (if applicable)	Briefly spin vial before opening; thoroughly resuspend powder (if applicable)
	Standard degraded	Store sample as recommended
	Curve doesn't fit scale	Try plotting using different scale
Low signal	Incubation time too short	Try overnight incubation at 4°C
	Target present below detection limits of assay	Decrease dilution factor; concentrate samples
	Precipitate can form in wells upon substrate addition when concentration of target is too high	Increase dilution factor of sample
	Using incompatible sample type (e.g. serum vs. cell extract)	Detection may be reduced or absent in untested sample types
	Sample prepared incorrectly	Ensure proper sample preparation/dilution

Problem	Cause	Solution
High background	Wells are insufficiently washed	Wash wells as per protocol recommendations
	Contaminated wash buffer	Make fresh wash buffer
	Waiting too long to read plate after adding STOP solution	Read plate immediately after adding STOP solution
Large CV	Bubbles in wells	Ensure no bubbles present prior to reading plate
	All wells not washed equally/thoroughly	Check that all ports of plate washer are unobstructed/wash wells as recommended
	Incomplete reagent mixing	Ensure all reagents/master mixes are mixed thoroughly
	Inconsistent pipetting	Use calibrated pipettes and ensure accurate pipetting
	Inconsistent sample preparation or storage	Ensure consistent sample preparation and optimal sample storage conditions (eg. minimize freeze/thaws cycles)
Low sensitivity	Improper storage of ELISA kit	Store all reagents as recommended. Please note all reagents may not have identical storage requirements.
	Using incompatible sample type (e.g. Serum vs. cell extract)	Detection may be reduced or absent in untested sample types

Technical Support

For all technical or commercial enquiries please go to:
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