ab108915

Urokinase type plasminogen activator Human Chromogenic Activity Assay Kit

Instructions for Use

For the rapid, sensitive, and accurate measurement of Urokinase-type plasminogen activator activity in various samples.

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

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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 remodelling in angiogenesis, atherogenesis, tumor cell metastasis, and ovulation. High level of uPA is a poor prognostic marker for aggressive breast cancer, aggressive prostate cancer, bladder cancer and gastric cancer.

ab108915 uPA Human Chromogenic Activity Assay Kit is developed to determine Human uPA activity in plasma, serum, cell culture, cell lysate, and tissue samples. The assay measures the ability of uPA to activate the plasminogen to plasmin in coupled or indirect assays that contain uPA, plasminogen, and a plasmin-specific synthetic substrate. The amount of plasmin produced is quantitated using a highly specific plasmin substrate releasing a yellow para-nitroaniline (pNA) chromophore. The change in absorbance of the pNA in the reaction solution at 405 nm is directly proportional to the uPA enzymatic activity.

2. Assay Summary

Prepare all reagents, samples and standards as instructed.

Add 60 µl of Assay Mix to each well

Add 20 µl of uPA Standard or testing samples per well and mix.

Add 10 µl of Plasmin Substrate to each well and mix gently.
Read the absorbance at 405 nm immediately.

Cover and incubate at 37°C after each reading.

Seal the plate with sealing tape. Incubate the plate at 37°C in a humid incubator to avoid drying the plate.

For HIGH uPA activity samples, read the absorbances at 405 nm every 10 minutes for 90 minutes.

For LOW uPA activity samples, read the absorbances at 405 nm every 1 hour for 6 hours.

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: 30 ml.
- uPA Standard: 1 vial Human high molecular weight uPA, calibrated against WHO 2nd International Standard (100 IU, lyophilized).
- Human Plasminogen (Lyophilized): 2 vials.
- Plasmin Substrate (Lyophilized): 2 vials.

4. Storage and Handling

•Store components of ab108915 at recommended temperatures, upon arrival, up to the expiration date.
•Store Standard, Plasminogen, and Plasmin Substrate at -20^{0}C.
•Store Microplate and Assay Diluent at 2-8^{0}C.
•Unused microplate wells may be returned to the foil pouch. Reseal along zip-seal.

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 3,000 x *g* for 10 minutes and assay. If necessary, dilute samples within the range 2X – 5X with Assay Diluent, and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA can also be used as an anticoagulant. Heparin is not recommended).
2. **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3,000 x g for 10 minutes and remove serum. Assay undiluted samples. If necessary, dilute samples within the range of 2X – 5X with

Assay Diluent and assay. 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 x *rpm* for 10 minutes at 4°C to remove debris. Collect supernatants and assay. If necessary, dilute samples within the range of 2X – 5X with Assay Diluent and assay. Store samples at -80°C. Avoid repeated freeze-thaw cycles.
4. **Cell Lysate:** The cultured cells are lysed and solubilized with 50 mM Tris-buffered saline (pH 8.0) containing 0.5% Triton X-100 or 0.1% Tween 20 on ice for 30 minutes. Centrifuge samples at 14000 rpm for 10 minutes. Collect fresh cell lysate. If necessary, dilute samples into Assay Diluent; optimal dilution factor should be determined depending on application needs. The undiluted samples can be stored at -80°C. Avoid repeated freeze-thaw cycles.
5. **Tissue:** Extract tissue samples with 50 mM Tris-buffered saline (pH 8.0) containing 0.5% Triton X-100 or 0.1% Tween 20 on ice for 30 minutes. Centrifuge samples at 14000 rpm for 20 minutes. Collect supernatant. If necessary, dilute samples into Assay Diluent; optimal dilution factor should be determined depending on application needs. Store remaining extract at -80°C. Avoid repeated freeze-thaw cycles.

Reagent Preparation:

1. **Plasminogen:** add 0.55 ml of reagent grade water to generate a 1x stock solution. Allow the reagent to sit for 10 minutes with gentle agitation prior to use.
2. **Plasmin Substrate:** Add 0.55 ml of reagent grade water to generate a 1x stock solution. Allow the reagent to sit for 10 minutes with gentle agitation prior to use.
3. **Standard Curve:** Reconstitute the uPA Standard (200 IU) with 2.5 ml of Assay Diluent to generate a standard stock solution of 40 IU/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions.

Store the reconstituted standard and reagents at -20°C and use within 30 days. Avoid repeated freeze-thaw cycles.

- **For high level uPA activity samples:** Prepare triplicate standard points by serially diluting from the standard stock solution (40 IU/ml) two-fold with equal volume of Assay Diluent to produce 20, 10, 5, 2.5, 1.25, 0.625 and 0.313 IU/ml solutions. Assay Diluent serves as the zero standard (0 IU/ml).

Standard Point	Dilution	[H. uPA] (IU/ml)
P1	1 part Standard (40 IU/ml) + 1 part Assay Diluent	20.000
P2	1 part P1 + 1 part Assay Diluent	10.000
P3	1 part P2 + 1 part Assay Diluent	5.000
P4	1 part P3 + 1 part Assay Diluent	2.500
P5	1 part P4 + 1 part Assay Diluent	1.250
P6	1 part P5 + 1 part Assay Diluent	0.625
P7	1 part P6 + 1 part Assay Diluent	0.313
P8	Assay Diluent	0.000

- **For low level uPA activity samples:** Dilute the standard stock solution (40 IU/ml) 1:200 with Assay Diluent to yield a solution of 0.2 IU/ml. Prepare triplicate standard points by serially diluting the standard solution (0.2 IU/ml) twofold with equal volume of Assay Diluent to produce 0.1, 0.05, 0.025, 0.013, and 0.006 IU/ml solutions. Assay Diluent serves as the zero standard (0 IU/ml).

Standard Point	Dilution	[H.uPA] (IU/ml)
P1	1 part Standard (40 IU/ml) + 99 parts Assay Diluent ↓ 1 part Standard (0.4 IU/ml) + 1 part Assay Diluent	0.200
P2	1 part P1 + 1 part Assay Diluent	0.100
P3	1 part P2 + 1 part Assay Diluent	0.050
P4	1 part P3 + 1 part Assay Diluent	0.025
P5	1 part P4 + 1 part Assay Diluent	0.013
P6	1 part P5 + 1 part Assay Diluent	0.006
P7	Assay Diluent	0.000

7. Assay Method

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 to avoid evaporation. Seal the plate with sealing tape at each step.

1. Remove excess microplate strips from the plate frame.

2. Assay Mix: At room temperature, freshly prepare the desired volume of the Assay Mix by combining the following reagents according to the assay numbers (n) plus one.

Assay Mix Reagents	**n=1**
Assay Diluent	50 µl
Plasminogen	10 µl

3. Add 60 µl of Assay Mix from above to each well.

4. Add 20 µl of uPA standard or Sample to each well

5. Add 10 µl of Plasmin Substrate to each well and mix gently.

6. Read the absorbance at 405 nm at zero minutes for background O.D.

7. Seal the plate with sealing tape. Incubate the plate at 37°C.

High uPA activity Samples: Read the absorbances at 405 nm every 10 minutes for 90 minutes.

Low uPA Activity Samples: Read the absorbances at 405 nm every hour for 6 hours

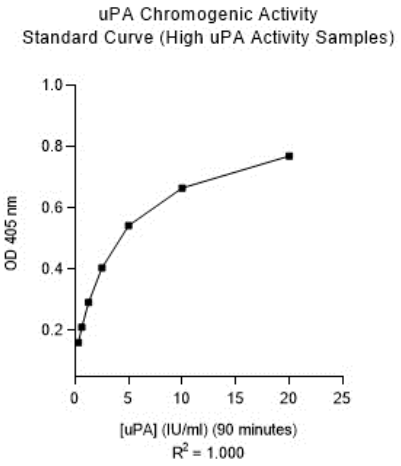
8. Data Analysis

- Calculate the mean value of the triplicate readings for each standard and sample.
- To generate a Standard Curve from the optimal 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 subtracting the background. The best-fit line can be determined by regression analysis of the 4-parameter curve.
- Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

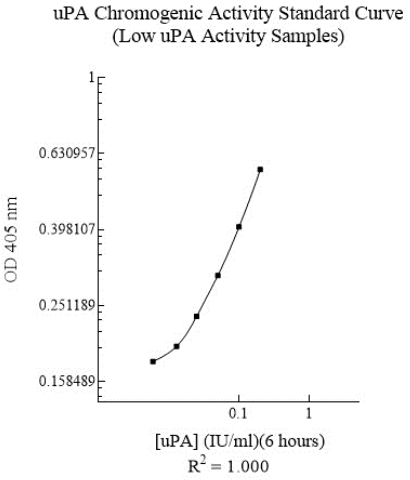
A. Typical Data

The curve is 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 samples



B. Sensitivity

Kit standard has been calibrated against WHO International Standard.

The minimum detectable dose of uPA at 6 hours is typically ~ 0.0051 IU/ml.

9. Specificity

No significant cross-reactivity or interference was observed.

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)