AssaySense Human uPA Chromogenic Activity Assay Kit (Indirect Assay)

Catalog Number CU1001b

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 (1, 2). High level of uPA is a poor prognostic marker for aggressive breast cancer, aggressive prostate cancer, bladder cancer and gastric cancer (3, 4, 5).

Principle of Assay

The AngioSense Human uPA Chromogenic Activity Assay Kit is developed to determine human uPA activity in plasma and cell culture supernatants. The assay measures the ability of uPA to activate the plasminogen to plasmin in coupled or indirect assays that contain uPA, plasminogen, and a plsmin-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.

Caution and Warning

- This kit is for research use only.
- The kit should not be used beyond the expiration date.
- All human source materials have been tested and found to be negative to HbsAg, HIV-1 and HCV by FDA approved methods.

Reagents

The activity assay kit contains sufficient reagents to perform 100 tests using microplate method.

- **Microplate:** one 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 (1200 IU)
- **Human Plasminogen:** 1 vial
- **Plasmin Substrate:** 2 vials

Storage Condition

- Store unopened kit at 2-8^{0}C up to expiration date.
- Opened reagents may be stored for up to 1 month at 2-8^{0}C. Store reconstituted standard and reagents at -20^{0}C or below.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 405 nm
- Pipettes (1-20 µl, 20-200 µl, and multiple channel)
- Deionized or distilled reagent grade water
- Incubator (37^{0}C)

Sample Collection, Preparation and Storage

- **Plasma:** Collect plasma using one-tenth volume of 0.11 M sodium citrate as an anticoagulant. Centrifuge samples at 2,000 x g for 10 minutes and assay. Samples can be stored at < -70^{0}C. Avoid repeated freeze-thaw cycles. Prior to the analysis dilute samples 1:5 into Assay Diluent.

- **Cell Culture Supernatants:** Centrifuge cell culture media at 3,000x g for 15 minutes at 4^{0}C to remove debris. Collect supernatants and assay. Samples can be store at < -70^{0}C. Avoid repeated freeze-thaw cycles. Prior to the analysis dilute samples 1:5 into Assay Diluent.

Reagent Preparation

- **Plasminogen:** Add 1.2 ml Assay Diluent.
- **Plasmin Substrate:** Add 1.1 ml reagent grader water.
- **Standard Curve:** Reconstitute the uPA Standard with 3 ml of Assay Diluent to generate a stock solution of 400 IU/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Dilute the stock solution 1:20 to generate a working solution of 20 IU/ml.

    - For high level of uPA activity samples, prepare triplicate standard points by serially diluting the standard working solution (20 IU/ml) twofold with equal volume of Assay Diluent to produce 10, 5, 2.5, 1.25 and 0.625 IU/ml.
    - For low level of uPA activity samples, dilute the working solution (20 IU/ml) 1:100 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, 0.0125, and 0.00625 IU/ml. Assay Diluent serves as the zero standard (0 IU/ml).

Standard curve for high level of uPA activity samples:

Standard Point	Dilution	[uPA] (IU/ml)
P1	1 part Standard (20 IU/ml)	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	Assay Diluent	0.000

Standard curve for low level of uPA activity samples:

Standard Point	Dilution	[uPA] (IU/ml)
P1	1 part Standard (0.2 IU/ml)	0.2000
P2	1 part P1 + 1 part Assay Diluent	0.1000
P3	1 part P2 + 1 part Assay Diluent	0.0500
P4	1 part P3 + 1 part Assay Diluent	0.0250
P5	1 part P4 + 1 part Assay Diluent	0.0130
P6	1 part P5 + 1 part Assay Diluent	0.0063
P7	Assay Diluent	0.0000

Assay Procedure

- Add 50 µl of the Assay Diluent to each well of the 96-well plate.
- Add 10 µl of the Plasminogen to each well.
- Add 20 µl of uPA Standards or testing samples per well and mix gently.
- Add 20 µl of Plasmin Substrate to each well and mix gently. Read the absorbance at 405 nm at zero minutes for background O.D. Seal the plate with sealing tape. Incubate the plate at 37^0C in a humid incubator to avoid drying the plate. For HIGH uPA activity samples, read the absorbances at 405 nm periodically every 5 minutes up to 30 minutes. For LOW uPA activity samples, start to read the absorbances at 405 nm from 3 hours up to 8 hours.

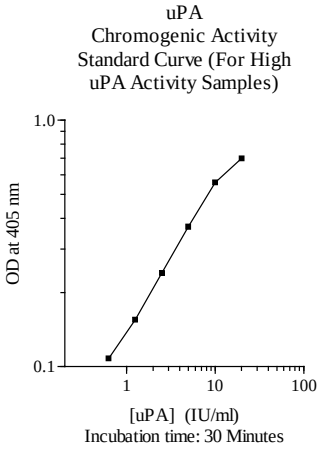
Assay Diluent	50 µl
Plasminogen	10 µl
uPA or Samples	20 µl
Plasmin Substrate	20 µl
High uPA activity Samples: *37^0C,* read the absorbances at 405 nm every 5 minutes for 30 minutes	
Low uPA Activity samples: *37^0C,* read the absorbances at 405 nm every hour from 3 hours to 8 hours	

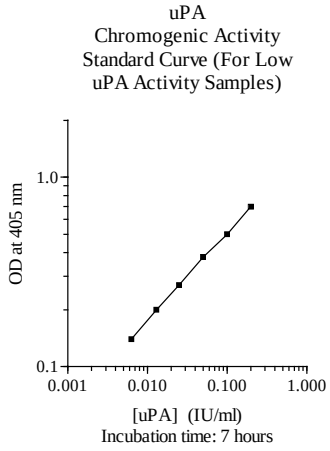
Data Analysis

- Calculate the mean value of the triplicate 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 (ΔA/min) on the y-axis after subtracting the background. 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 of 5.

Standard Curve

- The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.





Performance Characteristics

- The minimum detectable dose of uPA is typically < 0.006 IU/ml.
- No significant cross-reactivity or interference was observed.

References

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2. Besser, D. *et al.* (1996) *Fibrinolysis* 10: 215
3. Duffy, M.J. *et al.* (1990) *Cancer Res*. 50:6827
4. Hasui, Y. *et al.* (1992) *Int. J. Cancer* 50: 871
5. Nishino, N. *et al.* (1988) *Thromb. Res*. 50:527

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