AssaySense Human uPA Chromogenic Activity Assay Kit (Direct Assay)

Catalog Number CU1001a

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 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.

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)
- **uPA Substrate:** lyophilized substrate (2 tubes)

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

- **Substrate:** Add 1.1 ml reagent grade 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.

    - For high level of uPA activity samples, prepare triplicate standard points by serially diluting the standard solution (400 IU/ml) twofold with equal volume of Assay Diluent to produce 200, 100, 50, 25, 12.5 and 6.25 IU/ml.

    - For low level of uPA activity samples, dilute the stock solution (400 IU/ml) 1:32 with Assay Diluent to yield a solution of 12.5 IU/ml. Prepare triplicate standard points by serially diluting the standard solution (12.5 IU/ml) twofold with equal volume of Assay Diluent to produce 6.250, 3.125, 1.563, 0.781, and 0.391 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 (200 IU/ml)	200.00
P2	1 part P1 + 1 part Assay Diluent	100.00
P3	1 part P2 + 1 part Assay Diluent	50.00
P4	1 part P3 + 1 part Assay Diluent	25.00
P5	1 part P4 + 1 part Assay Diluent	12.50
P6	1 part P5 + 1 part Assay Diluent	6.25
P7	Assay Diluent	0.00

Standard curve for low level of uPA activity samples:

Standard Point	Dilution	[uPA] (IU/ml)
P1	1 part Standard (12.5 IU/ml)	12.500
P2	1 part P1 + 1 part Assay Diluent	6.250
P3	1 part P2 + 1 part Assay Diluent	3.125
P4	1 part P3 + 1 part Assay Diluent	1.562
P5	1 part P4 + 1 part Assay Diluent	0.781
P6	1 part P5 + 1 part Assay Diluent	0.391
P7	Assay Diluent	0.000

Assay Procedure

- Add 50 µl of the Assay Diluent to each well of the 96-well plate.
- Add 30 µl of uPA Standards or testing samples per well and mix gently.
- Add 20 µl of uPA Substrate to each well and mix gently. Seal the plate with a 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 2 hours up to 6 hours.

Assay Diluent	50 µl
uPA or Samples	30 µl
uPA Substrate	20 µl
High uPA activyt 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 2 hours to 6 hours	

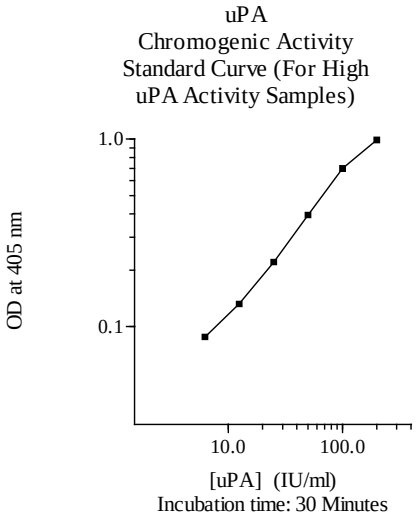
Data Analysis

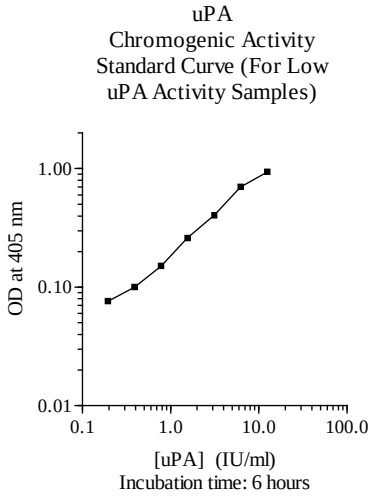
- Calculate the mean value of the triplicate for each standard and sample.

- 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. 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 < 0.1 IU/ml.
- No significant cross-reactivity or interference was observed.

References

1. Okada, S. *et al.* (1996) *Arterioscl. Thromb. Vasc. Biol*. 16: 1269
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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