Human uPA Chromogenic Activity Kit

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Hinweis/Note:
Der Packungsbeileger dient nur als erste Information.
Der relevante Packungsbeileger liegt der Ware bei.

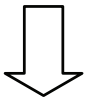
The datasheet is only a first information.
The relevant datasheet is included with the product.

For any questions regarding troubleshooting or performing the assay, please contact our support team at support@assaypro.com.

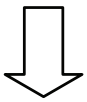
Thank you for choosing Assaypro.

Assay Summary

Add 50 µl of Assay Diluent per well.
Add 30 µl of Standard/ Sample per well.
Add 30 µl of Substrate per well.
Immediately read absorbance at 405 nm.



Incubate at 37°C.



High level uPA Activity samples: Read absorbance at 405 nm every 5 minutes up to 15 minutes.
Low level uPA Activity samples: Read absorbance at 405 nm every hour up to 6 hours.

Assay Template

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AssaySense Human uPA Chromogenic Activity Kit (Direct)

Catalog No. CU1001a
Sample Insert/Reference Only

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 processes including cell migration and tissue remodeling in angiogenesis, 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 (3-5).

Principle of Assay

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

Caution and Warning

- **Prepare all reagents as instructed, prior to running the assay.**
- **Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this protocol. However, the user should determine the optimal dilution factor.**
- 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 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Assay Diluent:** Ready to use, 30 ml.
- **Human uPA Standard:** Human high molecular weight uPA (50 IU, 1 vial).
- **Human uPA Substrate:** Lyophilized substrate (3 vials).

Storage Condition

- Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- Store uPA Substrate and Standard at -20°C.
- Store Microplate and Assay Diluent at 2-8°C.
- Unused microplate wells may be returned to the pouch and resealed.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 405 nm
- Pipettes (1-20 µl, 20-200 µl, 200-1000 µl, and multiple channel)
- Deionized or distilled reagent grade water
- Incubator (37°C)

Sample Collection, Preparation and Storage

- **Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x *g* for 10 minutes, use supernatants, and assay. If necessary, dilute samples within the range of 1x – 5x into Assay Diluent. 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).
- **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. If necessary, dilute samples within the range of 1x – 5x into Assay Diluent. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Cell Culture Supernatants:** Centrifuge cell culture media at 3000 x g for 10 minutes to remove debris and collect supernatants. If necessary, dilute samples into Assay Diluent and assay. The undiluted samples can be stored at -20°C or below. Avoid repeated freeze-thaw cycles.

Reagent Preparation

- **Human uPA Substrate:** Add 1.1 ml reagent grade water.

- **Standard Curve:** Reconstitute the Human uPA Standard (50 IU/ml) with 2 ml of Assay Diluent to generate a 25 IU/ml standard solution. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions.

    - For **high level** uPA activity samples, prepare duplicate or triplicate standard points by serially diluting the standard solution (25 IU/ml) 1:2 with equal volume of Assay Diluent to produce 12.5, 6.25, 3.125, 1.563, and 0.781 IU/ml solutions. Assay Diluent serves as the zero standard (0 IU/ml). Any remaining solution should be frozen at -20°C and used within 30 days.

    - For **low level** uPA activity samples, dilute the standard stock solution (25 IU/ml) 1:8 with Assay Diluent to yield a standard working solution of 3.125 IU/ml. Prepare duplicate or triplicate standard points by serially diluting the standard working solution (3.125 IU/ml) 1:2 with equal volume of Assay Diluent to produce 1.563, 0.781, 0.391, 0.195, and 0.098 IU/ml solutions. Assay Diluent serves as the zero standard (0 IU/ml). Any remaining solution should be frozen at -20°C and used within 30 days.

Standard curve for high level uPA activity samples:

Standard Point	Dilution	[uPA] (IU/ml)
P1	Standard (25 IU/ml)	25.00
P2	1 part P1 + 1 part Assay Diluent	12.50
P3	1 part P2 + 1 part Assay Diluent	6.250
P4	1 part P3 + 1 part Assay Diluent	3.125
P5	1 part P4 + 1 part Assay Diluent	1.563
P6	1 part P5 + 1 part Assay Diluent	0.781
P7	Assay Diluent	0.000

Standard curve for low level uPA activity samples:

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

Assay Procedure

- Prepare all reagents, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (25°C) for specific sample binding and at 37°C for chromogenic activity assay. Seal the plate with sealing tape at each step.
- Remove excess microplate strips from the plate frame.
- Add 50 µl of Assay Diluent to each well.
- Add 30 µl of Human uPA Standard or sample to each well and mix gently.
- Add 30 µl of Human uPA Substrate to each well and mix gently. Read the absorbance at 405 nm at zero minutes for background O.D. Seal plate with sealing tape and incubate at 37°C.
- For **high** level uPA activity samples, read the absorbance at 405 nm every 5 minutes up to 15 minutes.
- For **low** uPA activity samples, read the absorbance at 405 nm every hour up to 6 hours.

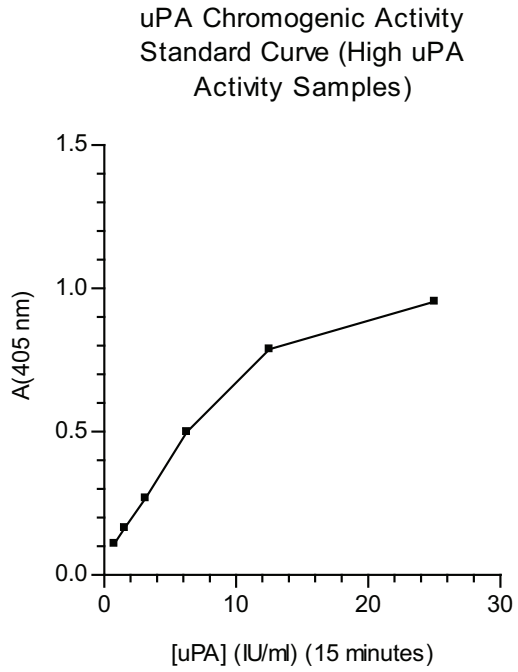
Assay Diluent	50 µl
uPA Standard or Sample	30 µl
uPA Substrate	30 µl
High level uPA activity samples: Incubate 37°C, read the absorbance at 405 nm every 5 minutes up to 15 minutes.	
Low level uPA activity samples: Incubate 37°C, read the absorbance at 405 nm every hour up to 6 hours.	

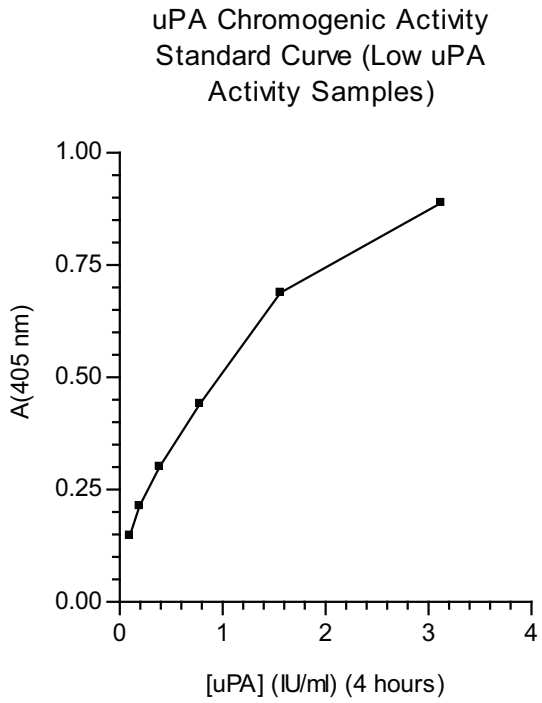
Data Analysis

- Calculate the mean value of the duplicate or 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 (ΔA/min) on the y-axis. The best-fit line can be determined by regression analysis using four-parameter or log-log lobistic curve-fit.
- Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

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 ~ 0.09 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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