Human uPA
Activity Kit

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

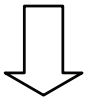
The datasheet is only a first information.
The relevant datasheet is included with the product.

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 20 µl of Standard/ Sample per well.
Add 60 µl of Assay Mix per well.
Add 10 µl of Plasmin Substrate per well.
Read at 405 nm at 0 minutes for background O.D.
Incubate at 37°C.



Read every 10 minutes for 1 hour (High Activity).
Read every 1 hour for 4 hours (Low Activity).

Assay Template

	A	B	C	D	E	F	G	H
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AssaySense Human uPA Indirect Chromogenic Activity Kit

Catalog No. CU1001b
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). A high level of uPA is a marker associated with a poor prognosis 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 supernatant 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.

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 diluent, 30 ml.
- **Human uPA Standard:** 1 vial, human high molecular weight uPA (30 IU, lyophilized)
- **Human Plasminogen:** 1 vial, lyophilized.
- **Plasmin Substrate:** 2 vials, lyophilized.

Storage Condition

- Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- Store Standard, Plasminogen, and Plasmin Substrate at -20°C.
- Store Microplate and Assay Diluent at 2-8°C.
- Unused microplate wells may be returned to the pouch and resealed.
- Opened Assay Diluent may be stored for up to 30 days at 2-8°C.

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. Assay undiluted samples (1x). 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 (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 and remove serum. Assay undiluted samples (1x). 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.

- **Cell Culture Supernatants:** Centrifuge cell culture media at 3000 x *g* for 15 minutes at 4°C to remove debris and collect supernatants. Dilute samples if necessary with Assay Diluent and assay. Samples can be stored at -20°C or below. Avoid repeated freeze-thaw cycles.

Reagent Preparation

- **Plasminogen:** Add 1.2 ml reagent grade water. Allow the reagent to sit for 10 minutes with gentle agitation prior to use.
- **Plasmin Substrate:** Add 0.55 ml reagent grade water. Allow the reagent to sit for 10 minutes with gentle agitation prior to use.
- **Standard Curve:** Reconstitute the 30 IU of Human uPA Standard with 1.5 ml of Assay Diluent to generate a 20 IU/ml standard solution. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Store reconstituted standard and reagents at -20°C or below and use within 30 days.
    - For **high level** of uPA activity samples, prepare duplicate or triplicate standard points by serially diluting the standard solution (20 IU/ml) 1:2 with Assay Diluent to produce 10, 5, 2.5, 1.25, 0.625, and 0.313 IU/ml solutions. Assay Diluent serves as the zero standard (0 IU/ml).
    - For **low level** of uPA activity samples, dilute the standard solution (20 IU/ml) 1:100 with Assay Diluent to yield a solution of 0.2 IU/ml. Prepare duplicate or triplicate standard points by serially diluting the standard solution (0.2 IU/ml) 1:2 with 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 curve for high level of uPA activity samples:

Standard Point	Dilution	[uPA] (IU/ml)
P1	Standard (20 IU/ml)	20.00
P2	1 part P1 + 1 part Assay Diluent	10.00
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

Standard curve for low level of uPA activity samples:

Standard Point	Dilution	[uPA] (IU/ml)
P1	1 part Standard (20 IU/ml) + 99 parts 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

Assay Procedure

- Prepare all reagents, working standards, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at 37°C for chromogenic activity assay. Seal the plate with sealing tape at each step.
- Remove excess microplate strips from the plate frame.
- 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.

Reagents	n=1
Assay Diluent	50 µl
Plasminogen	10 µl

- Add 60 µl of the above Assay Mix per well.
- Add 20 µl of Human uPA Standard or sample per well.
- Add 10 µl of Plasmin Substrate per 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°C.
- For high uPA activity samples, read the absorbance at 405 nm every 10 minutes for 1 hour.
- For low uPA activity samples, read the absorbance at 405 nm every 1 hour for 4 hours.

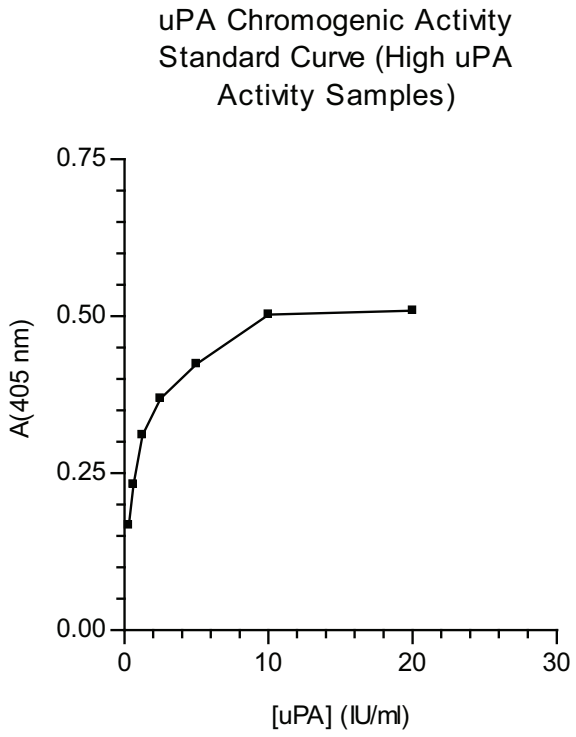
Assay Mix	60 µl
Standard or Sample	20 µl
Plasmin Substrate	10 µl
High uPA Activity samples: Incubate 37°C, read the absorbance at 405 nm every 10 minutes for 1 hour.	
Low uPA Activity samples: Incubate 37°C, read the absorbance at 405 nm every 1 hour for 4 hours.	

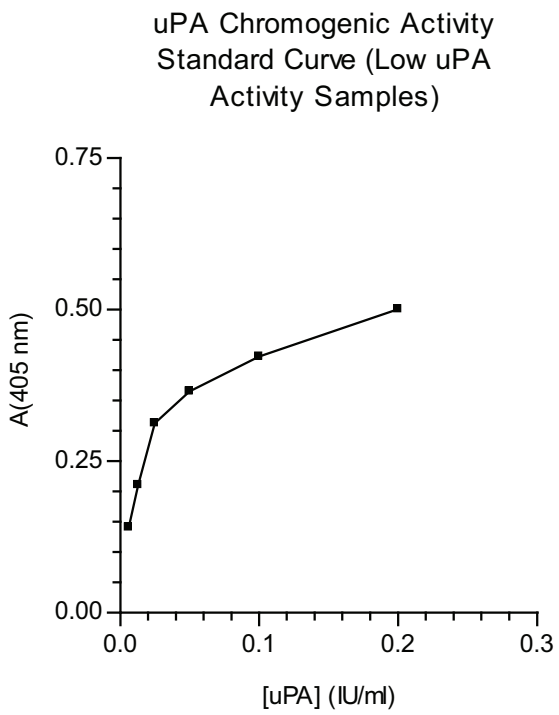
Data Analysis

- Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
- To generate a standard curve, 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.

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.

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

Related Products

- CU1001a Human uPA (Direct) Chromogenic Activity Kit (Plasma, Serum, and Cell Culture samples)

Version 2.9