AssaySense

Human uPA Chromogenic Activity Kit

Assaypro LLC
3400 Harry S Truman Blvd
St. Charles, MO 63301
T (636) 447-9175
F (636) 395-7419
www.assaypro.com

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

Step 1. Add 60 µl of Assay Mix per well.
Add 20 µl of Standard or Sample per well.
Add 10 µl of Plasmin Substrate per well.

Step 2. Read the absorbance at 405 nm for a zero minute background reading.
Cover and incubate at 37°C.

Step 3. Read every 10 minutes for 90 minutes (High Activity).
Read every 1 hour for 6 hours (Low Activity).
Cover and incubate at 37°C after each reading.

Symbol Key

Consult instructions for use.

Assay Template

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

Catalog No. CU1001b

Sample insert for reference use 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 the Assay

The AssaySense Human uPA Chromogenic Activity Kit is developed to determine uPA activity in human **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.

Caution and Warning

- This product is for **Research Use Only** and is not intended for use in diagnostic procedures.
- 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 insert. However, the user should determine the optimal dilution factor.
- The kit should not be used beyond the expiration date.

Reagents

The activity assay kit contains sufficient reagents to perform 96 tests using the microplate method.

- **Microplate:** A 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.
- **Human uPA Standard:** Human high molecular weight uPA, calibrated against WHO 2nd International Standard (100 IU, lyophilized).
- **Assay Diluent (1x):** Buffered protein base (30 ml).
- **Human Plasminogen:** Lyophilized, 2 vials.
- **Plasmin Substrate:** Lyophilized, 2 vials.

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.

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 and collect plasma. The sample is suggested for use at 1x or within the range of 2x – 5x into Assay Diluent; however, user should determine optimal dilution factor depending on application needs. 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).
- **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. The sample is suggested for use at 1x or within the range of 2x – 5x into Assay Diluent; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be

stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

- **Cell Culture Supernatant:** Centrifuge cell culture media at 1500 rpm for 10 minutes at 4°C to remove debris and collect supernatant. If necessary, dilute samples into Assay Diluent; user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -80°C. Avoid repeated freeze-thaw cycles.
- **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; user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -80°C. Avoid repeated freeze-thaw cycles.
- **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; user should determine optimal dilution factor depending on application needs. Store remaining extract at -80°C. Avoid repeated freeze-thaw cycles.

Applicable samples may also include biofluids, cell culture, and tissue homogenates. If necessary, user should determine optimal dilution factor depending on application needs.

Refer to Dilution Guidelines for further instruction.

Guidelines for Dilutions of 100-fold or Greater (*for reference only; please follow the insert for specific dilution suggested)*	
100x	**10000x**
A) 4 µl sample : 396 µl buffer (100x) = 100-fold dilution *Assuming the needed volume is less than or equal to 400 µl.*	A) 4 µl sample : 396 µl buffer (100x) B) 4 µl of A : 396 µl buffer (100x) = 10000-fold dilution *Assuming the needed volume is less than or equal to 400 µl.*
1000x	**100000x**
A) 4 µl sample : 396 µl buffer (100x) B) 24 µl of A : 216 µl buffer (10x) = 1000-fold dilution *Assuming the needed volume is less than or equal to 240 µl.*	A) 4 µl sample : 396 µl buffer (100x) B) 4 µl of A : 396 µl buffer (100x) C) 24 µl of B : 216 µl buffer (10x) = 100000-fold dilution *Assuming the needed volume is less than or equal to 240 µl.*

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use.
- **Human uPA Standard:** Reconstitute the Human uPA Standard (100 IU) with 2.5 ml of Assay Diluent to generate a 40 IU/ml standard stock solution. Allow the vial 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 from the standard stock solution (40 IU/ml) 2-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).
  - For **low level** uPA activity samples: From the standard stock solution (40 IU/ml), dilute 200-fold with Assay Diluent to produce a 0.2 IU/ml standard working solution. Prepare duplicate or triplicate standard points by serially diluting the standard working solution (0.2 IU/ml) 2-fold 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).

  Any remaining stock solution should be stored at -20°C and used within 30 days. Avoid repeated freeze-thaw cycles.

Standard curve for high level uPA activity samples:

Standard Point	Dilution	[uPA] (IU/ml)
P1	1 part Standard (40 IU/ml) + 1 part Assay Diluent	20
P2	1 part P1 + 1 part Assay Diluent	10
P3	1 part P2 + 1 part Assay Diluent	5.0
P4	1 part P3 + 1 part Assay Diluent	2.5
P5	1 part P4 + 1 part Assay Diluent	1.25
P6	1 part P5 + 1 part Assay Diluent	0.625
P7	1 part P6 + 1 part Assay Diluent	0.313
P8	Assay Diluent	0.0

Standard curve for low level uPA activity samples:

Standard Point	Dilution	[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	0.2
P2	1 part P1 + 1 part Assay Diluent	0.1
P3	1 part P2 + 1 part Assay Diluent	0.05
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	0.0

- **Plasminogen:** Add 0.55 ml of reagent grade water to generate a 1x stock solution. Allow the vial to sit for 10 minutes with gentle agitation prior to use. Any remaining stock solution should be stored at -20°C and used within 30 days. Avoid repeated freeze-thaw cycles.
- **Plasmin Substrate:** Add 0.55 ml of reagent grade water to generate a 1x stock solution. Allow the vial to sit for 10 minutes with gentle agitation prior to use. Any remaining stock solution should be stored at -20°C and used within 30 days. Avoid repeated freeze-thaw cycles.

Assay Procedure

- Prepare all reagents, standard solutions, 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.
- Remove excess microplate strips from the plate frame.
- Freshly prepare the desired volume of the Assay Mix by combining the following reagents according to the number of wells in the assay (n) plus one well.

Assay Mix Reagent	n = 1 well
Assay Diluent	50 µl
Plasminogen	10 µl

- Add 60 µl of Assay Mix to each well. Gently tap plate to thoroughly coat the wells. Add 20 µl of Human uPA Standard or sample to each well. Add 10 µl of Plasmin Substrate to each well. Gently tap plate to ensure thorough mixing. Break any bubbles that may have formed. Read the absorbance at 405 nm for a zero minute background reading. Cover wells with a sealing tape and incubate at 37°C in a humid incubator. Cover wells and incubate at 37°C after each reading.

- For **high level** uPA activity samples, read the absorbance at 405 nm every 10 minutes for 90 minutes.
- For **low level** uPA activity samples, read the absorbance at 405 nm every 1 hour for 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 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 (ΔA/min) 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.

Typical Data

- The typical data is provided for reference only. Individual laboratory means may vary from the values listed. Variations between laboratories may be caused by technique differences.

Standard data for high level uPA activity samples:

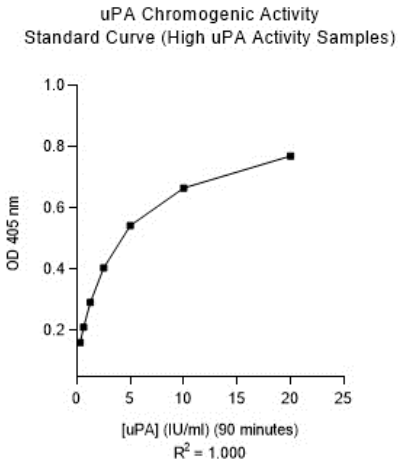
Standard Point	IU/ml	Average OD (90 min)
P1	20	0.769
P2	10	0.665
P3	5.0	0.542
P4	2.5	0.404
P5	1.25	0.292
P6	0.625	0.210
P7	0.313	0.161
P8	0.0	0.081

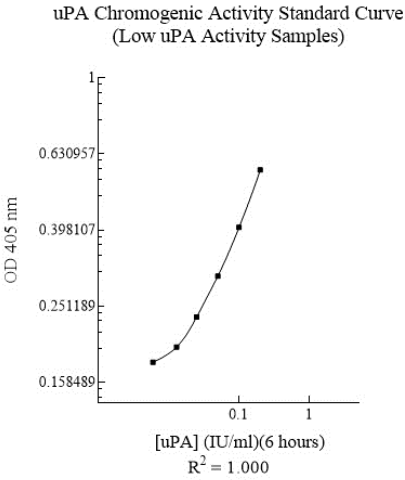
Standard data for low level uPA activity samples:

Standard Point	IU/ml	Average OD (6 hours)
P1	0.2	0.573
P2	0.1	0.404
P3	0.05	0.301
P4	0.025	0.235
P5	0.013	0.196
P6	0.006	0.179
P7	0.0	0.153

Standard Curve

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





Performance Characteristics

- **Kit standard has been calibrated against WHO International Standard**.
- The minimum detectable dose of human uPA at 6 hours is approximately 0.0051 IU/ml.

Troubleshooting

Issue	Causes	Course of Action
Low Precision	Use of improper components	• Check the expiration date listed before use. • Do not interchange components from different lots.
	Splashing of reagents while loading wells	• Pipette properly in a controlled and careful manner.
	Inconsistent volumes loaded into wells	• Pipette properly in a controlled and careful manner. • Check pipette calibration. • Check pipette for proper performance.
	Insufficient mixing of reagent dilutions	• Thoroughly agitate the lyophilized components after reconstitution. • Thoroughly mix dilutions.
Unexpectedly Low or High Signal Intensity	Microplate was left unattended between steps	• Each step of the procedure should be performed uninterrupted.
	Omission of step	• Consult the provided procedure for complete list of steps.
	Steps performed in incorrect order	• Consult the provided procedure for the correct order.
	Insufficient amount of reagents added to wells	• Check pipette calibration. • Check pipette for proper performance.
	Improper reagent preparation	• Consult reagent preparation section for the correct dilutions of all reagents.

	Insufficient or prolonged incubation periods	• Consult the provided procedure for correct incubation time.
Deficient Standard Curve Fit	Non-optimal sample dilution	• User should determine the optimal dilution factor for samples.
	Contamination of reagents	• A new tip must be used for each addition of different samples or reagents during the assay procedure.
	Contents of wells evaporate	• Verify that the sealing film is firmly in place before placing the assay in the incubator or at room temperature.
	Improper pipetting	• Pipette properly in a controlled and careful manner. • Check pipette calibration. • Check pipette for proper performance.
	Insufficient mixing of reagent dilutions	• Thoroughly agitate the lyophilized components after reconstitution. • Thoroughly mix dilutions.

References

(1) Okada S *et al*. (1996) *Arterioscl Thromb Vasc Biol*. 16:1269.
(2) Besser D *et al*. (1996) *Fibrinolysis*. 10:215.
(3) Duffy MJ *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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