AssaySense

Human tPA 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 80 µl of Assay Mix per well.
Add 20 µl of Standard or Sample 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 1 hour for 8 hours (High Activity).
Read every 1 hour from 20 hours up to 26 hours (Low Activity).

Symbol Key

Consult instructions for use.

Assay Template

	A	B	C	D	E	F	G	H
1								
2								
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AssaySense Human Tissue-type Plasminogen Activator (tPA) Chromogenic Activity Kit

Catalog No. CT1001

Sample insert for reference use only

Introduction

Tissue-type plasminogen activator (tPA) is a serine protease that converts the zymogen plasminogen into the active serine protease plasmin, the primary enzyme responsible for the removal of fibrin deposits (1). tPA is a 68 kDa glycoprotein that is synthesized by endothelial cells in normal blood vessels and displays relatively high affinity for fibrin, suggesting that it functions predominately in physiological thrombolysis in vivo (2). A high level of tPA is a good prognostic marker for breast cancer (3-4). tPA may minimize the formation of metastasis by preventing tumor cell adherence at sites of trauma (5). On the other hand, gastrointestinal cancer is accompanied by a decrease in tPA (6).

Principle of the Assay

The AssaySense Human tPA Chromogenic Activity Kit is developed to determine tPA activity in human **plasma and serum samples**. This kit is also validated for use with **canine, bovine, equine, monkey, mouse, rat, swine, and rabbit samples.** The assay measures the ability of tPA to activate the plasminogen to plasmin in coupled or indirect assays that contain tPA, 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 tPA 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 tPA Standard:** Lyophilized (128 IU).
- **Assay Diluent (1x):** Buffered protein base (30 ml).
- **Human Plasminogen:** Lyophilized, 3 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 acidified 0.5 M sodium citrate (pH 4.0) as an anticoagulant to prevent tPA-PAI complex formation. Centrifuge samples at 3000 x *g* for 15 minutes. To overcome interference by plasmin inhibitors, a 4-fold sample dilution is suggested into Assay Diluent; however, user should determine optimal dilution factor depending on application needs. **Incubate at room temperature for 10 minutes prior to loading assay**. The undiluted samples can be stored at -60°C or below. 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. To overcome interference by plasmin inhibitors, a 4-fold sample dilution is suggested into Assay Diluent; however, user should determine optimal dilution factor depending on application needs. **Incubate at**

room temperature for 10 minutes prior to loading assay. The undiluted samples can be stored at -60°C or below. 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 tPA Standard:** Reconstitute the Human tPA Standard (128 IU) with 0.8 ml of Assay Diluent to generate a 160 IU/ml standard stock solution. Allow the vial to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting from the standard stock solution (160 IU/ml) 4-fold with Assay Diluent to produce 40, 10, 2.5, 0.625, and 0.156 IU/ml solutions. Assay Diluent serves as the zero standard (0 IU/ml). Aliquot remaining stock solution to limit repeated freeze-thaw cycles. This solution should be stored at -20°C and **used within 10 days**.

Standard Point	Dilution	[tPA] (IU/ml)
P1	1 part Standard (160 IU/ml) + 3 parts Assay Diluent	40
P2	1 part P1 + 3 parts Assay Diluent	10
P3	1 part P2 + 3 parts Assay Diluent	2.5
P4	1 part P3 + 3 parts Assay Diluent	0.625
P5	1 part P4 + 3 parts Assay Diluent	0.156
P6	Assay Diluent	0.0

- **Plasminogen:** Add 1.7 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. Aliquot remaining stock solution to limit repeated freeze-thaw cycles. This solution should be stored at -20°C and **used within 48 hours**.
- **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	60 µl
Plasminogen	10 µl
Plasmin Substrate	10 µl

- Add 80 µl of Assay Mix to each well. Gently tap plate to thoroughly coat the wells. Add 20 µl of Human tPA Standard or sample 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 after each reading.
- For **high level** tPA activity samples, read the absorbance at 405 nm every 1 hour for 8 hours.
- For **low level** tPA activity samples, read the absorbance at 405 nm every 1 hour from 20 hours up to 26 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 (OD) 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.
- For **low level** tPA activity samples, it is recommended to start calculations from the lowest saturated point. Saturation may start to occur after 8 hours.
- Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

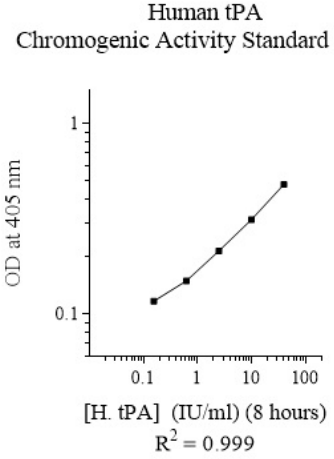
Typical Data

- 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 Point	IU/ml	Average OD
P1	40	0.479
P2	10	0.313
P3	2.5	0.214
P4	0.625	0.149
P5	0.156	0.117
P6	0.0	0.098

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 human tPA at 8 hours is approximately 0.13 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) Vassalli JD *et al*. (1991) *J Clin Invest*. 88:1067.
(2) Collen D, Lijnen HR. (1991) *Blood*. 78:3114.
(3) Duffy MJ *et al*. (1992) *Fibrinolysis*. 6:55.
(4) Ruppert C *et al*. (1997) *Cancer Detect Prev*. 21:452.
(5) Murthy MS *et al*. (1991) *Cancer*. 68:1724.
(6) Nishino N *et al*. (1988) *Thromb Res*. 50:527.

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