ab108905

Tissue type Plasminogen Activator Activity Assay Kit (Colorimetric, Human)

Instructions for Use

For the quantitative measurement of Human Tissue type Plasminogen Activator activity in plasma, serum and cell culture samples.

This product is for research use only and is not intended for diagnostic use.

Version 5c Last Updated: 30 September 2020

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1. Introduction

Tissue type Plasminogen Activator (tPA) is a 68 kDa serine protease that converts the zymogen plasminogen into the active serine protease plasmin which digests fibrin and induces the dissolution of fibrin clots. Tissue type Plasminogen Activator 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*. High level of Tissue type Plasminogen Activator is a good prognostic marker for breast cancer. On the other hand, gastrointestinal cancer is accompanied by a decrease in Tissue type Plasminogen Activator.

ab108905 Tissue type Plasminogen Activator Human Chromogenic Activity Assay kit is developed to determine Human Tissue type Plasminogen Activator activity in plasma, serum and cell culture samples. The assay measures the ability of Tissue type Plasminogen Activator to activate the plasminogen to plasmin in coupled or indirect assays that contain Tissue type Plasminogen Activator, 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 Tissue type Plasminogen Activator enzymatic activity.

2. Assay Summary

Prepare all reagents, samples and standards as instructed.

Add 80 µl Assay Mix to each well.

Add 20 µl Tissue type Plasminogen Activator standards or samples to each well.

Incubate at 37°C in a humid incubator.

For High Tissue type Plasminogen Activator samples read absorbance at 405 nm periodically for up to eight hours.

For Low Tissue type Plasminogen Activator samples read the absorbance at 405 nm from 20 to 26 hours.

3. Kit Contents

- Microplate: A 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.
- Human Tissue type Plasminogen Activator Standard: (1 vial).
- Diluent: (30 ml).
- Human Plasminogen: (1 vial)
- Plasmin Substrate: (2 vials).

4. Storage and Handling

Store components of the kit at 2-8°C or -20°C upon arrival up to the expiration date. Store Tissue type Plasminogen Activator Standard, Human Plasminogen and Plasmin Substrate at -20°C. Store Microplate and Diluent at 2-8°C. Opened unused microplate wells may be returned to the foil pouch with the desiccant packs. Reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator. Diluent (1x) may be stored for up to 1 month at 2-8°C.

5. Additional Materials Required

- Microplate reader capable of measuring absorbance at 405nm.
- Precision pipettes to deliver 1 µl to 1 ml volumes.
- Distilled or deionized reagent grade water.
- Humidified incubator at 37°C.

6. Preparation of Reagents

Sample Collection:

1. **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. Samples can be stored at < -60°C. Avoid repeated freeze-thaw cycles.
2. **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x g for 15 minutes and remove serum. A 4-fold sample dilution is suggested 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.

3. **Cell culture supernatants:** Centrifuge cell culture media at 3000 x *g* for 10 minutes at 4°C to remove debris. Collect supernatants and assay. Samples can be stored at <-60°C. Avoid repeated freeze-thaw cycles.

Reagent Preparation:

1. **Human Plasminogen:** Add 1.2 ml reagent grade water. Allow the plasminogen to sit for 15 minutes with gentle agitation prior to use; keep the vial on ice. Any remaining solution should be frozen at -2°C and used within 30 days.
2. **Plasmin Substrate:** Add 0.55 ml reagent grade water. Allow the plasmin substrate to sit for 15 minutes with gentle agitation prior to use; keep the vial on ice. Any remaining solution should be frozen at -20°C and used within 30 days.
3. **Standard Curve:** Reconstitute Tissue type Plasminogen Activator Standard with 0.4 mL Diluent to generate a standard solution of 40 IU/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare triplicate standard points by serially diluting the standard solution (40 IU/ml) 1:8 with equal volume of Diluent to produce 10, 2.5, 0.625, 0.156, and 0.039 IU/ml. Diluent serves as the zero standard (0 IU/ml) Any remaining solution should be frozen at -20°C and used within 30 days.

Standard Point	Dilution	[Tissue type Plasminogen Activator] (IU/ml)
P1	Standard (40 IU/ml)	40.000
P2	1 part P1 + 3 parts Diluent	10.000
P3	1 part P2 + 3 parts Diluent	2.500
P4	1 part P3 + 3 parts Diluent	0.625
P5	1 part P4 + 3 parts Diluent	0.156
P6	1 part P5 + 3 parts Diluent	0.039
P7	Diluent	0.000

7. Assay Method

1. Prepare all reagents, working standards and samples as instructed.
2. Freshly prepare the desired volume of Assay Mix by combining the following reagents as described below. The values represent the volumes for one reaction:

Diluent	60 µl
Plasminogen	10 µl
Plasmin Substrate	10 µl

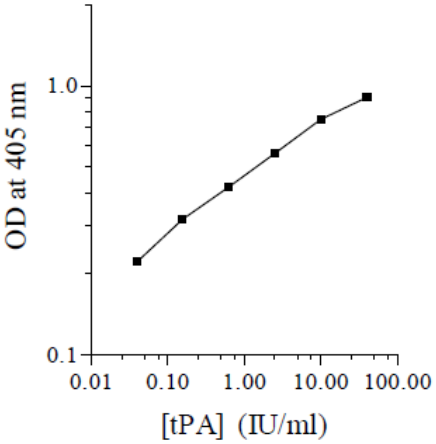
3. Add 80 µl of the Assay Mix to each well.
4. Add 20 µl of Tissue type Plasminogen Activator Standard or sample to each well and mix gently.
5. Read the absorbance at 405 nm at zero minutes for background O.D. Seal the plate with sealing tape. Incubate at 37°C in a humid incubator to prevent the plate drying out.
6. Read the absorbance on a microplate reader at a wavelength of 405 nm periodically as described below:
7. For High Tissue type Plasminogen Activator samples: read every hour for up to 8 hours.
8. For Low Tissue type Plasminogen Activator samples: start to read every hour from 20 hours for up to 26 hours.

8. Data Analysis

Calculate the mean value of the 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 of the linear portion of the curve. For Low Tissue type Plasminogen Activator 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.

A. Typical Data

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



B. Sensitivity

The minimum detectable dose of Tissue type Plasminogen Activator is typically 0.035 IU/ml.

9. Specificity

Cross-reactivity: 100% with mouse was observed.

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