

**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.

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# 1. Introduction

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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. Tissue type Plasminogen Activator 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*. A high level of Tissue type Plasminogen Activator is a good prognostic marker for breast cancer. Tissue type Plasminogen Activator may minimize the formation of metastasis by preventing tumor cell adherence at sites of trauma. 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

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Prepare all reagents, samples and standards as instructed.



Add 80  $\mu$ l Assay Mix to each well.



Add 20  $\mu$ l 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

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- 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: (3 vials).
- Plasmin Substrate: (2 vials).

### 4. Storage and Handling

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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

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- 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

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### 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. 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.

### **Reagent Preparation:**

1. **Human Plasminogen:** Add 1.2 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.
2. **Plasmin Substrate:** Add 0.55 ml reagent grade water to generate a 1X stock solution. 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 0.3 mL Assay Diluent to generate a 160 IU/ml standard stock solution. 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 (160 IU/ml) 1:4 (with 3 parts Diluent to 1 part Standard Point), to produce 40, 10, 2.5, 0.625, and 0.039 IU/ml solutions. 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.

<b>Standard Point</b>	<b>Dilution</b>	<b>[Tissue type Plasminogen Activator] (IU/ml)</b>
P1	1 part Standard (160 IU/ml) + 3 parts Diluent	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	Assay Diluent	0.0



## 7. Assay Method

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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 $\mu$ l
Plasminogen	10 $\mu$ l
Plasmin Substrate	10 $\mu$ l

3. Add 80  $\mu$ l of the Assay Mix to each well.
4. Add 20  $\mu$ l 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 after each reading.
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.

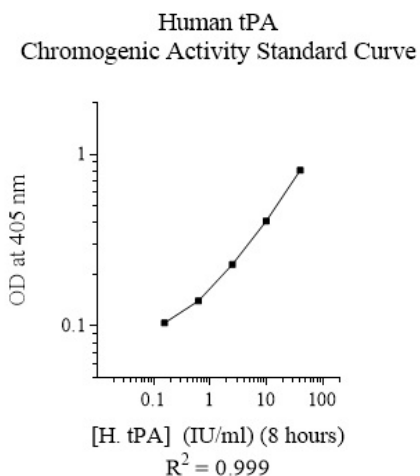
## 8. Data Analysis

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Calculate the mean value of the 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 ( $\Delta A/\text{min}$ ) 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 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 0.13 IU/ml.

## 9. Specificity

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This kit is validated for use with canine, bovine, equine, monkey, mouse, rat, swine, and rabbit samples.

### Technical Support

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