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Plasminogen Activator Inhibitor-1 Activity Assay Kit (C)

03/20

(Catalog # K2040-100; 100 assays; Store at -20°C)

I. Introduction:

Plasminogen Activator Inhibitor-1 (PAI-1), also known as endothelial plasminogen activator inhibitor is a serine protease inhibitor that functions as the principal inhibitor of tissue plasminogen activator (tPA) and urokinase (uPA). PAI-1 is present in a number of tissues & cell types and is associated with various thrombotic and fibrinolytic complications. High level of PAI-1 activity is found in patients suffering from myocardial infarction, hemolytic uremic syndrome or stroke whereas low levels of PAI-1 activity are associated with bleeding disorders. Additionally, PAI-1 is a prognosticator in various cancers. **BioVision's Plasminogen Activator Inhibitor-1 Activity Assay Kit** is a two-step colorimetric assay. In the first step, samples are incubated with a known amount of tPA thereby allowing PAI-1 and tPA to form an inactive complex. In the second step, the residual free tPA converts plasminogen to plasmin, which in turn hydrolyzes the plasmin chromogenic substrate thereby releasing *p*NA (chromophore). The absorbance of the released *p*NA is inversely proportional to the PAI-1 activity in the samples. The assay kit is simple, specific and can detect as low as 2 U/ml of PAI-1 activity in samples.

PAI-1 + tPA ⟶ PAI-1•tPA + **Residual tPA**

Plasminogen + Substrate Mix —(**Residual tPA**)⟶ Cleaved Substrate + *p*NA **(Absorbance, OD 405 nm)**

II. Applications:

- Measurement of PAI-1 activity in Biological Samples such as plasma, cell culture medium
- Analysis and study of fibrinolytic system

III. Sample Type:

- Biological Fluids such as plasma
- Cell Culture Supernatants such as EA.hy926

IV. Kit Contents:

Components	K2040-100	Cap Code	Part Number
PAI-1 Assay Buffer	25 ml	WM	K2040-100-1
PAI-1 Acidify Buffer	5 ml	NM/Red	K2040-100-2
PAI-1 Stop Buffer	5 ml	NM/Blue	K2040-100-3
Human tPA	1 vial	Green	K2040-100-4
Plasminogen	1 vial	Yellow	K2040-100-5
PAI-1 Substrate Mix	1 vial	Red	K2040-100-6
Clear 96-well half area plate	1 plate	--	K2040-100-7

V. User Supplied Reagents and Equipment:

- Multi-well spectrophotometer
- dH_2O

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay. Upon opening, use within two months.

- **PAI-1 Assay Buffer, PAI-1 Acidify Buffer & PAI-1 Stop Buffer:** Store at either 4°C or -20°C. Bring to room temperature (RT) before use.
- **Human tPA:** Reconstitute with 100 µl PAI-1 Assay Buffer to prepare 5800 U/ml Human tPA stock solution. Pipette up and down to mix well. Divide into aliquots and store at -20°C. Keep on ice while in use.
- **Plasminogen:** Reconstitute the vial with 44 µl PAI-1 Assay Buffer to prepare the Plasminogen stock solution. Store at -20°C. Keep on ice while in use.
- **PAI-1 Substrate Mix:** Reconstitute the vial with 120 µl dH_2O. Divide into aliquots and store at -20°C. Keep on ice while in use.
- **Clear 96-well half area plate:** Upon receiving, store the plate at RT.

VII. Plasminogen Activator Inhibitor-1 Activity Assay Protocol:

1. 40 U/ml tPA Solution Preparation:

Prepare a 145-fold dilution of the Human tPA stock solution with PAI-1 Assay Buffer to make **40 U/ml tPA solution** (i.e. add 2 µl of Human tPA stock solution into 288 µl PAI-1 Assay Buffer). Keep on ice while in use.

2. Sample, "0 U/ml PAI-1" & "40 U/ml PAI-1" Solution Preparation:

Plasma: Collect the citrate treated human plasma. Centrifuge the plasma at 5,000 x *g* for 10 min and 4°C to prepare the platelet poor plasma. Collect the supernatant and centrifuge again at 5,000 x *g* and 4°C for another 10 min. Collect the supernatant, which is the prepared plasma for the subsequent treatments. Store the prepared plasma at 2-8°C while in use. If not used immediately, divide into aliquots and store the prepared plasma immediately at -70°C.

Cell Culture Supernatant: Grow the endothelial cells to confluency (usually 1-2 days). Collect the cell culture medium and centrifuge at 3,000 x *g* for 15 min and 4°C to remove any debris. Collect the cell supernatant.

Mix 20 µl of prepared plasma or cell supernatant with 20 µl of 40 U/ml tPA in an eppendorf tube labeled as **"Sample"**. Mix 20 µl of 40 U/ml tPA with 20 µl of PAI-1 Assay Buffer in an eppendorf tube labeled as **"0 U/ml PAI-1"**. Add 40 µl of PAI-1 Assay Buffer in another eppendorf tube labeled **as "40 U/ml PAI-1"**. Incubate all the three eppendorf tubes at RT for 20 min.

	Sample	40 U/ml tPA	PAI-1 Assay Buffer
Sample	20 µl	20 µl	--
0 U/ml PAI-1	--	20 µl	20 µl
40 U/ml PAI-1	--	--	40 µl

Notes:

a. Citrate treated platelet poor plasma must be used for the assay. Platelet contamination may cause spurious results.
b. PAI-1 is unstable and must be processed and frozen within 3 hr of specimen collection.
c. There is large diurnal variation of PAI-1 activity in plasma, which should be taken into consideration when designing clinical studies and routine applications. It is recommended that specimens should be the early morning fasting specimen.
d. If PAI-1 activity is above 40 U/ml, dilute the sample(s) with PAI-1 Assay Buffer and mark the dilution factor.

3. **Acidification & Neutralization Step:** Add 40 µl of PAI-1 Acidify Buffer to all three eppendorf tubes including **Sample**, **0 U/ml PAI-1** and **40 U/ml PAI-1** (from Step 2). Mix well and incubate at 37°C for 20 min, protected from light. Add 80 µl of PAI-1 Assay Buffer to all the three tubes and mix well. The "**Sample"** tube is now ready for the assay. Add 10 µl of the Sample into two parallel wells of a clear 96-well half area plate designed as **"PAI-1 Sample"** and **"PAI-1 Sample Background Control"**.

Note: Equilibrate the clear 96-well half area plate to 37°C before adding the Sample(s).

4. **Standard Curve Preparation:**

a. **Intermediate "PAI-1" Standard Preparation:** Prepare various intermediate PAI-1 Standards including 10 U/ml, 20 U/ml and 30 U/ml using PAI-1 Acidify Buffer treated 0 U/ml PAI-1 and 40 U/ml PAI-1 (from Step 3) according to the table below. Mix well.

	0 U/ml PAI-1	40 U/ml PAI-1
10 U/ml PAI-1	45 µl	15 µl
20 U/ml PAI-1	30 µl	30 µl
30 U/ml PAI-1	15 µl	45 µl

b. Add 10 µl of 0 U/ml PAI-1, 10 U/ml PAI-1, 20 U/ml PAI-1, 30 U/ml PAI-1 and 40 U/ml PAI-1 Standards (from Step 3 & 4) into wells of clear 96-well half area plate.

5. **Reaction Mix Preparation:** Prepare a 25-fold dilution of the Plasminogen stock solution with PAI-1 Assay Buffer (i.e. add 4 µl of Plasminogen stock solution with 96 µl PAI-1 Assay Buffer). Prepare 10-fold dilution of the reconstituted Substrate Mix with PAI-1 Assay Buffer (i.e. add 10 µl of reconstituted Substrate Mix with 90 µl PAI-1 Assay Buffer).

Prepare Reaction Mix (for both Standard(s) & PAI-1 Sample wells) and Background Mix (for PAI-1 Sample Background Control wells) according to the table below. Make sufficient amount of each type of mix to add 40 µl to all assay wells of that type.

	Reaction Mix	Background Mix
Diluted Plasminogen	10 µl	-- µl
Diluted Substrate Mix	10 µl	10 µl
PAI-1 Assay Buffer	20 µl	30 µl

Mix well. Add 40 µl of Reaction Mix to PAI-1 Standard(s) & PAI-1 Sample wells and 40 µl of Background Mix to PAI-1 Sample Background Control well(s). Mix well and incubate at 37°C for 90 min, protected from light. The total volume of each well is 50 µl. After 90-min incubation, add 50 µl of PAI-1 Stop Buffer to all wells containing PAI-1 Sample(s), PAI-1 Sample Background Control and PAI-1 Standards. Mix well.

Note: Equilibrate the PAI-1 Stop Buffer to 37°C before adding to the wells.

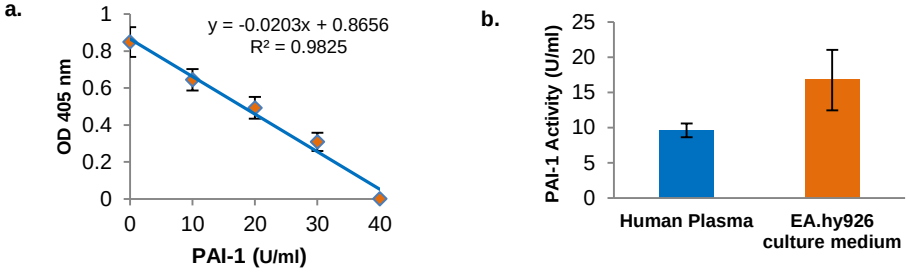
6. **Measurement:** Measure the colorimetric signal (OD 405 nm) at 37°C in end-point mode.

7. **Calculation:** Subtract the "40 U/ml PAI-1" Standard reading from all Standards readings. Plot the PAI-1 Standard Curve. Subtract the PAI-1 Sample Background Control reading from all PAI-1 Sample readings to get the corrected PAI-1 Sample readings. Apply the corrected PAI-1 Sample readings to the PAI-1 Standard Curve to obtain the corresponding PAI-1 activity (U/ml) as:

Sample PAI-1 Activity = B * D = U/ml

Where: **B** = PAI-1 activity from the Standard Curve (U/ml)
D = Sample dilution factor (D=1 for undiluted Sample(s))

Unit Definition: One unit of PAI-1 activity was defined as the amount of PAI-1 that inhibits one unit of tPA activity under Assay conditions.



Figures: a. PAI-1 Standard Curve. b. Measurement of PAI-1 Activity in pooled human plasma (citrate treated platelet poor plasma) and EA.hy926 culture medium. All assays were performed following kit protocols.

VIII. Related Products:

Tissue Plasminogen Activator Activity Assay Kit (K178)
Urokinase Activity Fluorometric Assay Kit (K728)
Plasmin Activity Assay Kit (Fluorometric) (K381)
Plasmin Activity Assay Kit (Colorimetric) (K945)

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