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Plasminogen (PLG) Assay Kit (Colorimetric)

11/20

(Catalog # K2064-100; 100 assays; Store at -20 °C)

I. Introduction:

Plasminogen (PLG) is the inactive precursor of plasmin, a serine protease that degrades many blood plasma proteins including fibrin clots. PLG is processed to plasmin by endogenous activators including tissue plasminogen activator, tPA or urokinase plasminogen activators (uPA) or exogenous activators such as streptokinase. PLG is synthesized in the liver and is mainly present in plasma at a stable concentration of 200 µg/ml. Plasmin play a key role in the degradation of fibrin clots and several constituents of the extracellular matrix. PLG is a central component of the fibrinolytic system and could be a vital contributor in various diseases. Hereditary plasminogen deficiency is a predisposing risk factor for thromboembolic disease whereas acquired PLG deficiency is often seen in severe liver diseases, acute disseminated intravascular coagulation etc. PLG activators have been used in therapeutics and are also known as "clot buster". **BioVision's Plasminogen Assay Kit** is a simple, plate based assay for measuring plasminogen levels in biological samples including plasma. In this assay, excess streptokinase is first mixed with plasminogen to form plasminogen-streptokinase complex. The complex then cleaves a specific, synthetic plasmin substrate releasing *p*NA. The released *p*NA is quantified by measuring absorbance at 405 nm. The colorimetric signal is directly proportional to the amount of plasminogen in samples. The assay is specific and high-throughput adaptable. The kit can detect as low as 0.2 µg/ml of plasminogen in Samples.

Plasminogen + Streptokinase ⟶ **Plasminogen-Streptokinase (Plasmin)**

Plasmin Substrate —(**Plasmin**)⟶ Cleaved Substrate + *p*NA (**Absorbance; OD 405 nm**)

II. Applications:

- Measurement of Plasminogen in biological samples such as Plasma
- Analysis and study of fibrinolytic system

III. Sample Type:

- Biological Fluids such as plasma

IV. Kit Contents:

Components	K2064-100	Cap Code	Part Number
PLG Assay Buffer	35 ml	NM	K2064-100-1
PLG Stop Buffer (10X)	20 ml	WM	K2064-100-2
Streptokinase	1 vial	Green	K2064-100-3
Fibrinogen (Plasminogen Free)	300 µl	Blue	K2064-100-4
Plasmin Substrate	400 µl	Red	K2064-100-5
Plasminogen Standard	1 vial	Yellow	K2064-100-6

V. User Supplied Reagents and Equipment:

- Multi-well spectrophotometer
- 96-well clear plate with flat bottom
- Deionized water

VI. Storage Conditions and Reagent Preparation:

Upon arrival, store the kit at -20 °C, protected from light. Briefly centrifuge all small vials prior to opening. Read the entire protocol before performing the assay.

- **PLG Assay Buffer and PLG Stop Buffer (10X):** Store at either 4 ºC or -20 ºC. Bring the buffers to room temperature (RT) before use.
- **Streptokinase:** Reconstitute the vial in 110 µl dH_2O to prepare the Streptokinase stock solution. Pipette up and down to mix well. Divide into aliquots and store at -20 °C. Keep on ice while in use.
- **Fibrinogen (Plasminogen Free):** Ready to use. Divide into aliquots and store at -20 ºC. Keep on ice while in use.
- **Plasmin Substrate:** Ready to use. Store at -20 °C, protected from light. Keep on ice while in use.
- **Plasminogen Standard:** Reconstitute the vial in 40 µl **PLG Assay Buffer** to prepare 1 mg/ml Plasminogen Standard stock solution. Divide into aliquots and store at -20 °C. Keep on ice while in use. Use within two months.

VII. Plasminogen Assay Protocol:

1. **Sample Preparation:**

   Plasma: Collect whole blood using citrate tubes. Centrifuge the plasma at 2,000-3,000 x *g* for 15 min. Collect the supernatant and centrifuge for another 15 min at 2,000-3,000 x *g* to deplete the platelets in the Plasma Sample(s). Collect the supernatant and keep the Sample(s) on ice, while handling. **Note:** If not used immediately, divide into aliquots and store the prepared platelet depleted plasma at -70 °C. Prepare a 20-fold dilution of the platelet depleted plasma sample in PLG Assay Buffer. Add 2-20 µl of diluted plasma into duplicate wells of a 96-well clear plate labeled as **Sample** and **Sample Background Control.** Adjust the volume to 20 µl/well with PLG Assay Buffer. Add 20 µl of PLG Assay Buffer to a well labeled as **Reagent Background Control**.

   Note: Platelet contamination may cause spurious results. Citrate treated (platelet poor) plasma is recommended for the assay.

2. **Standard Curve Preparation:** Prepare 1:50 dilution (20 ng/µl) of the Plasminogen Standard stock (1 mg/ml) solution by adding 2 µl of the 1 mg/ml Plasminogen Standard into 98 µl PLG Assay Buffer and mix well. Add 0, 2, 4, 6, 8 10 µl of 20 ng/µl Plasminogen Standard

into a series of wells to generate 0, 40, 80, 120, 160, 200 ng/well Plasminogen Standard respectively. Adjust the volume of all wells to 20 µl/well with PLG Assay Buffer.

3. **Reaction Mix Preparation:** Prepare a 10-fold dilution of Streptokinase stock solution (i.e. dilute 10 µl of Streptokinase stock solution with 90 µl PLG Assay Buffer) and mix well. Prepare a 10-fold dilution of Fibrinogen (i.e. dilute 10 µl Fibrinogen with 90 µl PLG Assay Buffer) and mix well. Mix enough reagents for the number of assays to be performed. For each well, prepare 60 µl **Reaction Mix** containing the following components. Mix well before use.

	Reaction Mix
PLG Assay Buffer	40 µl
Diluted Streptokinase	10 µl
Diluted Fibrinogen	10 µl

Add 60 µl of the Reaction Mix to each well containing PLG Standard(s), Sample (s), and Reagent Background Control. Add 60 µl PLG Assay Buffer to the Sample Background Control well. Mix well and **incubate at 37 °C for 10 min**, protected from light. *The total volume of each well is 80 µl*. **Note:** If original Fibrinogen is cloudy, centrifuge at 2,000 x *g* for 20 min at 4 °C and take the supernatant for assay.

4. **Substrate Hydrolysis:** Prepare 1X PLG Stop Buffer by mixing 100 µl of 10X PLG Stop Buffer with 900 µl dH_2O and mix well. Mix enough reagents for the number of Samples and Standards to be assayed. For each well, prepare 20 µl PLG Substrate Mix containing:

	PLG Substrate Mix
PLG Assay Buffer	16 µl
PLG Substrate	4 µl

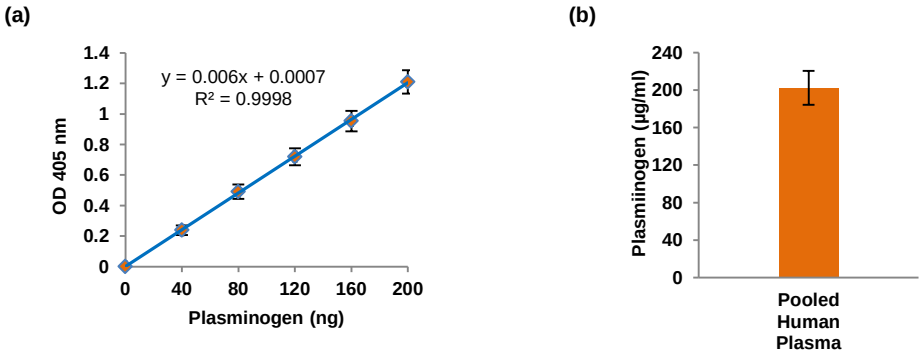
Add 20 µl of **PLG Substrate Mix** to all wells containing PLG Standard(s), Sample(s), Reagent Background Control and Sample Background Control. Mix well and **incubate at 37 °C for 10 min**, protected from light. After 10 min incubation, add 50 µl of 1X PLG Stop Buffer to all wells containing PLG Standard(s), Sample(s), Reagent Background Control and Sample Background Control. Mix well. *The final volume in each well should be 150 µl.* **Note:** Equilibrate the 1X PLG Stop Buffer to 37 °C prior to the assay.

5. **Measurement:** Measure the absorbance immediately at 405 nm at 37 °C in end point mode. The reaction is stable for at least 4 hr.

6. **Calculation:** Subtract 0 Standard reading from all Standards readings. Plot the Plasminogen Standard Curve. Subtract Sample Background Control reading from its paired Sample reading(s) to get corrected Sample reading. **If the Reagent Background Control reading is higher than the Sample Background Control reading, subtract the Reagent Background Control reading from the Sample reading instead**. Apply the corrected Sample reading to the PLG Standard Curve to get B ng of Plasminogen in the Sample.

Plasminogen amount in Sample = B/V * D = ng/ µl = µg/ml

Where: **B** is the PLG amount from the Plasminogen Standard Curve (ng)
V is the sample volume added into the reaction well (µl)
D is the Dilution factor (D = 1, for undiluted samples)



Figures. (a). Plasminogen Standard Curve. **(b).** Measurement of plasminogen amounts in pooled human platelet poor plasma (10 µl, 1:20 dilution). All assays were performed following the kit protocol.

VIII. RELATED PRODUCTS:

Plasminogen, Human Plasma (7549)
Plasminogen (PLG) (Human) ELISA Kit (E4641)
Plasminogen Activator Inhibitor-1 Activity Assay Kit (K2040)
Plasmin Activity Assay Kit (Fluorometric) (K381)
Plasmin Activity Assay Kit (Colorimetric) (K945)
Plasmin, Human Plasma (4089)
Plasminogen (PLG) (Rat) ELISA Kit (E4642)
Tissue Plasminogen Activator (tPA) Activity Assay Kit (K178)
Plasmin Inhibitor Screening Kit (Fluorometric) (K382)
Urokinase Activity Fluorometric Assay Kit (K728)

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