ab303727 – Plasminogen (PLG) Activity ELISA Kit (Colorimetric)

For the quantitative measurement of plasminogen in various biological samples.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab303727

Storage and Stability
Store the kit at -20 °C for up to 12 months, protected from light.

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". **The 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**)

Materials Supplied

Components	
PLG Assay Buffer	35 ml
PLG Stop Buffer (10X)	20 ml
Streptokinase	1 vial
Fibrinogen (Plasminogen Free)	300 µl
Plasmin Substrate	400 µl
Plasminogen Standard	1 vial

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Multi-well spectrophotometer
- 96-well clear plate with flat bottom
- Deionized water
- Multichannel pipette
- Microcentrifuge tube.

Reagent Preparation

- Briefly centrifuge all small vials at a low speed prior to opening
- Read the entire protocol before performing the experiment.

PLG Assay Buffer and PLG Stop Buffer (10X): Store at either 4 °C or -20 °C. Bring the buffers to room temperature (RT) before use.

Streptokinase: Reconstitute the vial in 110 µl dH2O 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 °C. 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.

Standard Preparation

1. 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.
2. 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.

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.

Assay Procedure

- It is recommended that all standards and samples be run at least in duplicate.

- Prepare all reagents, Standards and sample(s) as instructed
- Prepare enough reagents for the number of assays to be performed
- A standard curve should be run for each assay.

Reaction Mix Preparation:

1. 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
Diluteed Streptokinase	10 µl
Diluted Fibrinogen	10 µl

2. 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.

Substrate Hydrolysis:

3. Prepare 1X PLG Stop Buffer by mixing 100 µl of 10X PLG Stop Buffer with 900 µl dH2O 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

4. 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.

Measurement

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

Calculations

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)

For technical support contact information, visit: www.abcam.com/contactus