QuantiChrom™ Plasmin Assay Kit (DPLM-100)

Quantitative Colorimetric Assay for Plasmin Activity

DESCRIPTION

PLASMIN (3.4.21.7) is a serine protease that is a key enzyme in the process of fibrinolysis. Plasmin is released into circulation as its inactive form, plasminogen, which can be activated by enzymes such as urokinase. The plasmin/plasminogen system has been studied for its role in inflammation, degradation of the extracellular matrix, and wound healing. BioAssay Systems' DPLM-100 kit provides a convenient colorimetric method to measure plasmin activity. In this assay, plasmin hydrolyzes a synthetic substrate to release *p*-nitroanilide (*p*NA), which absorbs at 405 nm. The increase in absorbance at 405 nm is directly proportional to the enzyme activity.

KEY FEATURES

Safe and sensitive. Non-radioactive assay. Use as little as 10 µL samples. Linear detection range in 96-well plate: 0.35 to 56.5 U/L activity.

Fast and convenient. The procedure involves addition of a single working reagent and incubation for 30 min.

High-throughput. Homogeneous "mix-incubate-measure" type assay. Can be readily automated to assay thousands of samples per day.

APPLICATIONS

For quantitative determination of plasmin enzyme activity in biological samples.

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Assay Buffer: 10 mL **Substrate:** 200 µL

***p*NA Standard:** 50 µL 100 mM standard

Storage conditions. The kit is shipped at room temperature. Store all components at -20°C upon receiving. Shelf life: 6 months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

This assay can be run at room temperature or 37°C. Prior to assay, equilibrate Assay Buffer to desired temperature. Briefly centrifuge tubes before use. This assay is based on a kinetic reaction. To ensure identical incubation time, addition of Working Reagent to samples should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended.

Sample Preparation:

Note: Plasminogen is not measured by this assay. To measure plasminogen with this assay, samples must first be incubated with an activator of plasminogen, such as urokinase or streptokinase. This is not provided with the kit.

Serum can be assayed directly.

Standard Preparation: Prepare a 3000 µM Standard Premix by combining 6 µL of 100 mM pNA Standard and 194 µL of Assay Buffer. Prepare all standards according to table below.

No.	Premix + Assay Buffer	Total Volume (µL)	Std (µM)
1	100 µL + 0 µL	100 µL	3000
2	60 µL + 40 µL	100 µL	1800
3	30 µL + 70 µL	100 µL	900
4	0 µL + 100 µL	100 µL	0

Standards: Transfer 10 µL of each Standard dilution to separate wells of a clear 96-well plate.

Reaction Preparation:

1. Transfer 10 µL of each sample to separate wells.
2. Prepare enough Working Reagent (WR) for all *sample* wells by mixing, for each well, 2 µL Substrate and 95 µL Assay Buffer. Fresh reconstitution of WR is recommended. Keep WR at 37°C until it is added to sample wells.
3. To each *Standard* well (Standards #1 – 4), add 90 µL Assay Buffer. To each *Sample* well, add 90 µL WR. Tap plate briefly to mix.
4. Read OD_{405nm} immediately (OD_0) on a plate reader. Incubate protected from light at 37°C for 30 min, then read OD_{405nm} again (OD_{30}). Alternatively, after step 3, record kinetics at OD_{405nm} at 37°C for at least 30 min.

CALCULATION

Using the OD values from 30 min, subtract the blank value (Standard #4) from the standard values and plot ΔOD against the standard concentrations. Determine the slope (μM^{-1}) of the *p*NA standard curve and calculate the plasmin activity in each sample as follows:

$$\text{Plasmin Activity} = \frac{OD_{30} - OD_0}{\text{Slope } (\mu M^{-1}) \times t\,(\text{min})} \times n \ \text{(U/L)}$$

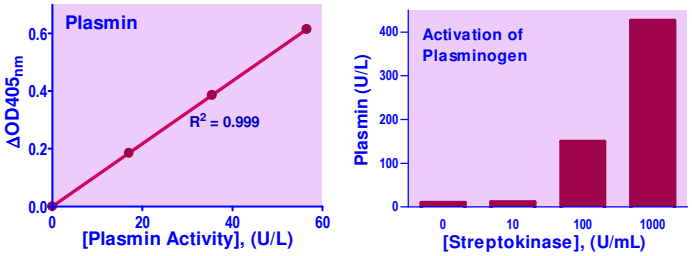
where OD_{30} and OD_0 are the OD_{405nm} values at 30 min and 0 min, respectively. *t* is the reaction time (30 min). *n* is the dilution factor.

Unit definition: 1 Unit (U) of plasmin will catalyze the release of 1 µmole of pNA per min at pH 7.4.

Note: If sample plasmin activity exceeds 56.5 U/L, either use a shorter reaction time or dilute samples in water and repeat the assay. If kinetics are recorded, any two time points in which the activity remains linear can be chosen for analysis. Use OD values from the latter time point to calculate the slope from the standards and adjust t *to the chosen time interval.*

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices and accessories (e.g. multi-channel pipettor), clear flat-bottom 96-well plates (e.g. VWR cat# 82050-760), centrifuge tubes and plate reader.



Left: Plasmin samples were assayed in a 96-well plate at 37°C.
Right: Plasminogen in human serum samples was activated by streptokinase after incubating for 30 min at 37°C.

LITERATURE

1. Baker, S. K., & Strickland, S. (2020). A critical role for plasminogen in inflammation. The Journal of experimental medicine, 217(4), e20191865.
2. Politis, I., Zavizion, B., Barbano, D. M., & Gorewit, R. C. (1993). Enzymatic Assay for the Combined Determination of Plasmin Plus Plasminogen in Milk: Revisited. Journal of Dairy Science, 76(5), 1260–1267.
3. Korycha-Dahl, M., Dumas, B., Chêne, N., & Martal, J. (1983). Plasmin activity in milk. Journal of Dairy Science, 66(4), 704–711.

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