PAI Activity Assay Kit

Cat. No. ECM610

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

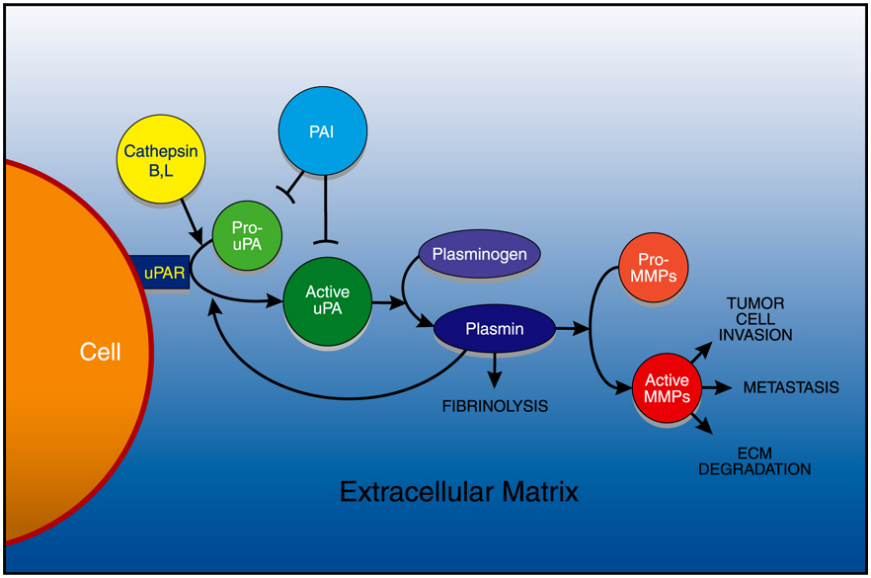
USA & Canada
Phone: +1(800) 437-7500 • Fax: +1 (951) 676-9209 • Europe +44 (0) 23 8026 2233
Australia +61 3 9839 2000 • Germany +49-6192-207300 • ISO Registered Worldwide
www.Millipore.com • custserv@Millipore.com • techserv@Millipore.com

Introduction

Plasminogen activator inhibitors (PAI)[1] are members of the serine protease inhibitor (serpin) superfamily and central regulatory protein in the blood coagulation system. These inhibitor acts as "bait" for urokinase-type and tissue-type plasminogen activators (uPA and tPA) [2,3] and protein C. PAIs are unique among serpins in exhibiting distinct active and inactive (latent) conformations in vivo. Human PAI-1 is a single-chain glycoprotein with a molecular weight of 43 kDa. Its rapid interaction with tPA may function as a major control point in the regulation of fibrinolysis. The highly mobile reactive-center loop (RCL) is thought to account for both the rapid inhibition of plasminogen activators, and the rapid and spontaneous transition of the unstable, active form of PAI-1 into the stable, inactive conformation (t1/2 at 37°C, 2 hours). The inactive form can be partially reactivated by denaturants such as urea, guanidine hydrochloride or SDS[4]. High concentrations of PAI-1 have been associated with human thromboembolic disease[5]. PAI-1 activity may limit the extent of tumor metastasis, since uPA activity is a major contributory factor promoting dissolution of tumor matrix and basement membrane. Human PAI-2 is a major product of monocytes and macrophages in response to inflammatory mediators. PAI-2 is synthesized in either an intracellular, nonglycosylated form with a molecular mass about 47 kDa or an extracellular, glycosylated form with a molecular mass of about 60 kDa. The relative distribution of intracellular and secreted forms of PAI-2 seems to depend on the cell type, culture conditions and cell differentiation state.

uPA is a 52 kDa serine protease that has been implicated in a number of physiological and pathological processes, including tissue remodeling[6], angiogenesis[7], fibrinolysis and tumor spread. When bound to its cell surface receptor, uPA is converted from the single chain pro-form uPA to the active 2-chain HMW-uPA. uPA has been shown to play a role in basement membrane degradation, via a cascade involving activation of plasminogen and the matrix metalloproteinases[8]. Inhibitors of uPA have been shown to slow primary tumor growth and metastasis[9-12].

The Millipore PAI Activity Assay Kit provides a quick, efficient and sensitive system for evaluation of inhibition of uPA activity by PAI and for screening of uPA inhibitors. The assay is colorimetric and does not require radioactivity or fluorescence equipment. The assay is sensitive over an inhibition range of 0.05-50 units of uPA activity.



Test Principle

The Millipore PAI Activity Assay Kit utilizes a chromogenic substrate, which is cleaved by active uPA, detectable by its Optical Density at 405nm (OD405). Addition of PAI sample blocks the cleavage of substrate by uPA.

Application

The Millipore PAI Assay Kit is ideal for measurement of PAI inhibitory activity toward uPA in purified preparations and cell culture, as well as in serum where pathological conditions such as sepsis exist. The assay is also useful for screening inhibitors of uPA.

Each Millipore PAI Activity Assay Kit contains sufficient reagents for the evaluation of 96 samples, including uPA standard from human urine and E. coli expressed recombinant protein corresponding to amino acids 24 to 427 of human PAI-1 (mature form) as positive control. PAI-1 positive control contains a mixture of the active and latent forms, with $\geq$ 45% of active form. Duplicate or triplicate samples are suggested.

The Millipore PAI Activity Assay Kit is intended for research use only; not for diagnostic or therapeutic applications.

Kit Components

1. uPA Positive Control: (Part No. 90058) One lyophilized vial, 1000 units, of uPA from human urine.
2. Chromogenic Substrate: (Part No. 90057) One 5 mg bottle of Tripeptide with pNA group.
3. Assay Buffer, 10X: (Part No. 90091) One 5 mL bottle.
4. PAI-1 Positive Control: (Part No. 90093) One 400 µL vial containing 20 µg of purified PAI-1 with 0.1% BSA.

Materials Not Supplied

1. Single or Multichannel Pipette and disposable tips
2. Microplate reader (405 nm)
3. 37°C incubator
4. Clean 96-well microplate for performing incubations.
5. Activation Buffers

PAI-1 Activation

1. PAI-1 positive control is provided at a concentration of 0.050 µg/µL with 0.1% BSA. The PAI-1 positive control exists in both an active and latent form. To fully activate the latent PAI-1, the PAI-1 standard must be incubated with Activation Buffer. (2X Activation Buffer: 8M Guanidine HCl, 40mM Sodium Acetate, pH 5.6, 400mM NaCl, 0.2% Tween 20)[13].
2. To activate the PAI-1 positive control, remove the desired amount of PAI-1 from the standard tube. It is recommended that you activate the entire tube then aliquot and freeze the PAI-1 standard.
3. Combine the PAI-1 sample with an equal volume of the 2X Activation Buffer. The PAI-1 concentration is now 0.025 µg/µL in the Activation Buffer.
4. Incubate the PAI-1 activation mixture at 37°C for 30 minutes.
5. Remove the Guanidine HCl from the PAI-1 mixture by dialyzing the sample against 1X Activation Buffer without Guanidine HCl (20mM sodium acetate, pH 5.6, 200mM NaCl, 0.1% Tween 20). Perform the dialysis at 4°C for at least 4 hours.

6. After dialysis, centrifuge the activated PAI-1 sample and proceed to the Assay Instructions. You can aliquot and freeze the activated PAI-1 sample for later use.

Assay Instructions

1. Rehydrate uPA positive control with 1 mL deionized H_2O. Following rehydration, aliquot and freeze at -70°C. Thaw PAI-1 positive control on ice, aliquot and freeze immediately at -70°C.
2. To each well of a clean 96-well plate, add 10-150 µL of activated PAI-containing sample or PAI-1 positive control to each well, depending on expected PAI levels.
3. Add sufficient deionized water to bring total volume to 150 µL.
4. Add 20 µL of the Assay Buffer and 10 µL of uPA positive control solution to each well. Incubate 15-30 min at 37°C.
5. Rehydrate the Chromogenic Substrate with 2 mL deionized H_2O. Store reconstituted substrate at 2° to 8°C.
6. Add 20 µL of the Chromogenic Substrate to each well.
7. Incubate at 37°C for 2 hours. Note: Longer incubation is required for samples with low PAI levels.
8. Read Absorbance on a standard microplate reader at 405 nm.

Standard Curve

The following table shows volumes to be used to make a standard curve with the enclosed PAI-1 positive control. This table should be used as a reference only.

PAI Pos. Control (µL)	uPA Pos. Control (µL)	Deionized H_2O (µL)	Assay Buffer (µL)	Assay Substrate (µL)	Total Volume (µL)
80	10	70	20	20	200
40	10	110	20	20	200
20	10	130	20	20	200
10	10	140	20	20	200
5	10	145	20	20	200
2.5	10	147.5	20	20	200
1.25	10	148.75	20	20	200
0.0	10	150	20	20	200

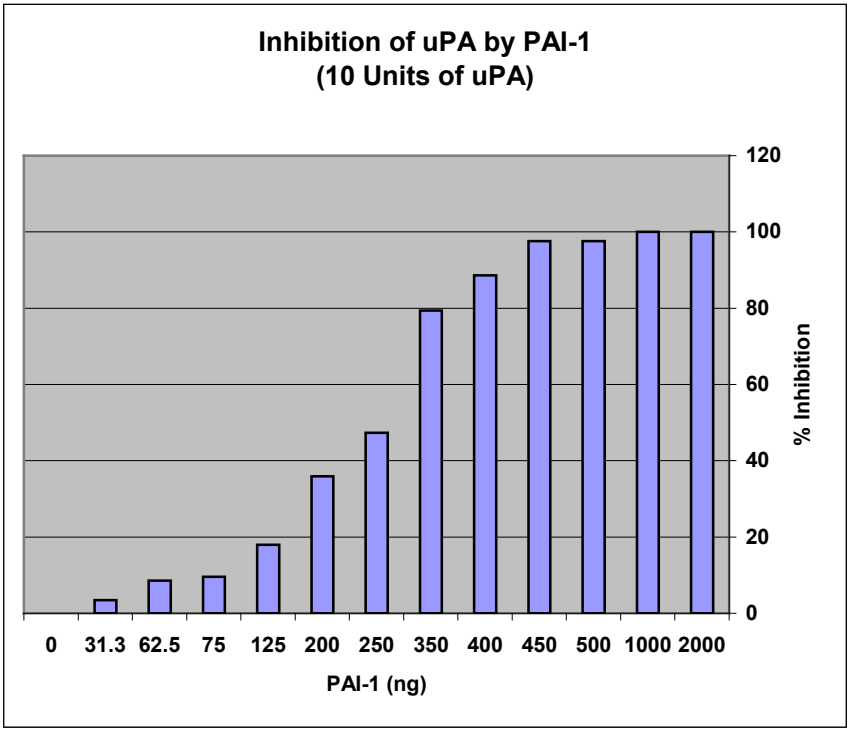
Storage

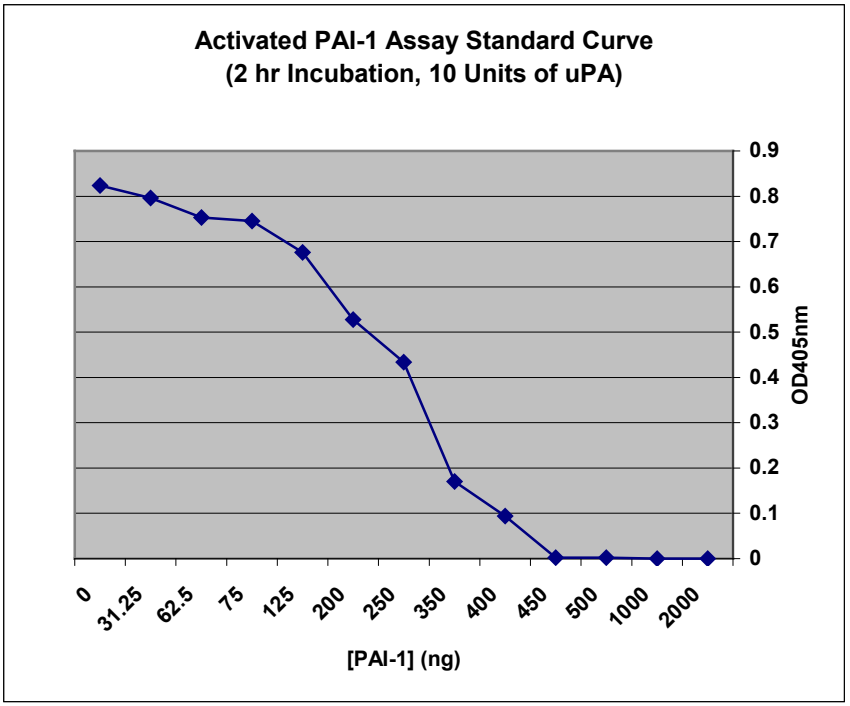
Store kit materials at -20°C for up to 12 months. Store reconstituted chromogenic substrate at 2° to 8°C.

Calculation of Results

Optical Density values obtained with the **Millipore** PAI Activity Assay Kit may be compared with known standards or other test samples to obtain relative activities. PAI-1 Positive Control provided with the kit is useful for quantitative purposes and it contains a mixture of the active and latent forms, with $\geq$ 45% of active form. Recombinant PAI-1 protein without carrier BSA is available separately as Cat. No. CC4075.

The following charts illustrate typical results upon dilution of the PAI-1 Positive Control contained in the kit. One should use the data below for reference only. This data should not be used to interpret actual assay results.





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