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BIOMEDICA DIAGNOSTICS

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monoclonal antibody against human PAI-1

REF 3785

Description

Plasminogen Activator Inhibitor Type-1 (PAI-1), a primary regulator of fibrinolysis, has been found in a number of different tissues, and cell types including macrophages/monocytes, hepatocytes, vascular endothelia, adipose tissue of the heart and lungs, and in platelets. The clinical interest in measuring PAI-1 in plasma is based on case studies in which levels of this serine protease inhibitor are associated with various thrombotic and fibrinolytic complications. Deficiency of PAI-1 activity is associated with bleeding disorders wherein the routine haemostatic screening tests are normal. High levels of PAI-1 activity are found in patients suffering from myocardial infarction, haemolytic uremic syndrome, and stroke. Levels of PAI-1 in the plasma of pregnant women are also correlated with gestational diabetes, reduced placental blood flow and preeclampsia. Patients with cirrhosis may also have elevated levels of PAI-1.

REF 3785 is a murine IgG_{1k} monoclonal antibody monoclonal antibody recognizing human PAI-1. It reacts with both active and latent PAI-1, and tPA/PAI-1 complexes, while demonstrating no reactivity with human PAI-2 or human PAI-3. REF 3785 has not been epitope mapped.[1]

Hazards and Precautions

For Reseach Use Only

Warning

Hazard Statement:	H319	Causes serious eye irritation.
Precautionary Statements:	P264	Wash thoroughly after handling.
	P280	Wear protective gloves/eye protection/face protection.
	P305 + P351 + P338	If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing
	P337 + P313.	If eye irritation persists: Get medical advice/attention.

Preparation

REF 3785 is purified from mouse ascites fluid via ammonium sulphate precipitation followed by Protein G affinity chromatography. Active PAI-1 secreted by the human melanoma cell line MJZJ was used as the immunizing antigen.

Presentation

Screw-capped glass vial containing 0.5 mg of purified IgG_{1k} lyophilized from a 0.5 mL solution of 0.15M Phosphate Buffered Saline, with 0.02% sodium azide added.

Reconstitution

Add 0.5 mL of filtered deionized or sterile water to generate a 1.0 mg/mL stock solution.

Storage

Store lyophilized antibody at 2°-8°C. Aliquot and store reconstituted antibody at –20°C or colder.

Applications

Immunohistochemistry

At concentrations ranging from 10-50 µg/mL, REF 3785 has reliably stained PAI-1 in both frozen and formalin-fixed, paraffin embedded tissue sections of human breast cancer, cardiac vascular tissue, coronary artery, gastric epithelia, lung and epidermal (normal and scar) tissue.[2-10]

Western blot

REF 3785 is useful for Western blots of human PAI-1. An antibody concentration of 5 µg/mL is recommended.[11]

Inhibition of PAI-1 Activity

While in-house studies have demonstrated an ability for REF 3785 to inhibit the activity of a mutant human PAI-1 in a direct tPA activity assay, no inhibitory effect was found in a tPA/plasmin generation based-chromogenic assay.[12]

References

1. Björquist, P. *et al.* *Biochemica et Biophysica Acta* 1997, **1341**: 87-98.
2. Jankun, J., *et al.* *Journal of Cellular Biochemistry*, **53**: 135-144, 1993.

3. Costantini, V., *et al. Cancer* 1996, **77**: 1079-1088.
4. Sobel, B. E., *et al. Circulation* 1998, **97**: 2213-2221.
5. Tuan, T., *et al. American Journal of Pathology* 2003, **162**: 1579-1589.
6. Hildenbrand, R. and Arens, N. *British Journal of Cancer* 2004, **91**: 564-571.
7. Cederqvist, K., *et al. Pediatrics* 2005, **117**: 1226-1234.
8. Koensgen, D., *et al. Anticancer Research* 2006, **26**: 1683-1690.
9. Beyer, B. C. M., *et al. Cancer* 2006, **106**: 1026-1035.
10. Kenny, S., *et al. American Journal of Physiology Gastrointestinal Liver Physiology* 2008, **295**: G431-G441.
11. Shiratsuchi, T., *et al. Journal of Cellular Physiology* 2002, **193**: 340-348.
12. Marshall, L. J., *et al. The Journal of Immunology* 2003, **171**: 2057-2065.

Definition of Symbols

	Consult instructions for use
	Manufacturer
	Refer to Safety Data Sheet
	Temperature Limitation
LOT	Lot Number
	Expiration Date
REF	Catalog Number

Immunohistochemical Staining of PAI-1 in Paraffin Embedded Tissue Sections Using Murine Monoclonal Antibody No. 3785

A) Dewaxing

1. Dewax tissue section in a glass staining dish with xylene for 10 minutes. Repeat in a second dish.

B) Rehydration and Blocking

2. Block endogenous peroxidase activity with 0.3% H_2O_2 in methanol for 10 minutes.
3. Rehydrate tissue section with successive dilutions of ethanol at room temperature for 10 minutes. Start with 100% ethanol and dilute to 95%, 75%, 50% and finally 25% with PBS.
4. Block tissue section with 10% normal goat serum at 37°C for 30 minutes. Wash tissue 3 times with PBS.

C) Staining

5. Incubate tissue section with MAb No. 3785, 40 µg/mL (1:25 dilution) in assay solution at 37°C for 60 minutes.
6. Wash with assay solution 3 times, 5 minutes each wash.
7. Incubate with HRP conjugated goat anti-mouse IgG at 37°C for 30 minutes.
8. Wash with assay solution 3 times, 5 minutes each wash.
9. Incubate tissue sections with substrate solution for 2-10 minutes. Check sections after 2 minutes. Do not overdevelop.
10. Wash with distilled water 1-3 times, 5 minutes each wash.
11. Counterstain if desired, clear and mount in appropriate medium.

Assay Solution:	0.15M PBS, 1% BSA, 0.3% Tween 20, pH 7.4
Blocking Solution:	10% Normal Goat Serum in Assay Solution
Substrate Solution:	3, 3' - Diaminobenzidine, dissolved in 50 mM Tris, pH 7.4 to 1 mg/mL. Add an equal amount of 0.03% H_2O_2 immediately prior to use.

Procedural Notes

1. Optional - Increased sensitivity may be obtained by treating tissue section with 0.1% Trypsin in PBS at 37°C for 30 minutes, followed by PBS wash, 3 times, 5 minutes each. This treatment is performed between steps 3 and 4 (rehydration and preimmune serum block).
2. Do not include sodium azide as an anti-bacterial agent in the perparation of any solution used with peroxidase staining procedures. Azide inhibits peroxidase activity.
3. Diaminobenzidine is a suspected carcinogen. Handle with caution, wear disposable gloves.
4. All incubations are performed at 37°C. Do not let tissue sections dry at any time during paraffin removal or staining procedure.
5. Goat sera for blocking and goat secondary antibody are suggested. Any preimmune sera with corresponding species second antibody can be used.