

Catalog # B1400

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GPC3 (HUMAN GLYPICAN3) IMMUNOHISTOCHEMISTRY KIT

Intended Use

The BioMosaics Inc. GPC3 Immunohistochemistry Kit is a histochemical staining kit for the detection and localization of GPC3 in human tissue sections. Suitable for paraffin or frozen sections.

This assay is for research use only and not for use in diagnostic or therapeutic procedures.

Storage of Kit Components

Kit components are shipped on blue ice. Upon receipt, store entire kit at 4-8 C. Kit is stable until expiration date is stored according to manufacturers' recommendations.

Materials Provided*

The following is the list of components which are included:

- 1 Blocking Solution (2.5% horse serum)
- 2 1G12 Detector Antibody (ready to use)
- 3 Anti-Mouse Ig HRP Conjugate Reagent
- 4A DAB Concentrate
- 4B DAB Reaction Buffer
- 5 Hematoxylin Counterstain
- 6 Mounting Media

** Positive Control Slides are available separately (Cat No. C0032)*

Materials Required But Not Provided

- Hydrogen peroxide (30% solution) for quenching endogenous peroxidase activity
- Phosphate buffered saline (PBS) solution
- Distilled water
- Ethyl alcohol
- Xylene
- Coverslips
- Methanol

Staining Protocol

1. Deparaffinization (FOR PARAFFIN-EMBEDDED TISSUES ONLY)

Note: If you are not using paraffin-embedded tissues, skip to step 2 below. If paraffin-embedded tissues are used, it is necessary to deparaffinize the slides before following the staining protocol below.

Deparaffinization involves incubation of the slides in xylene followed by a graded alcohol series as follows:

Xylene	5 Minutes, then change to new coplin jar containing Xylene
Xylene	5 Minutes
100% ethyl alcohol	5 Minutes
90% ethyl alcohol	3 Minutes
80% ethyl alcohol	3 Minutes
70% ethyl alcohol	3 Minutes
PBS	3 Minutes

2. Staining

Component and/or Procedure	Component Preparation	Procedure	Time
Quenching Solution (not provided)	Quenching Solution Dilute 30% hydrogen peroxide 1:10 in methanol.	Immerse slides into coplin jars filled with quenching solution for 10 minutes. Wash with PBS x1 for 2 minutes	10 minutes
Antigen Retrieval	10 mmol citrate buffer pH 6.0 (not provided)	Heat Slides in 10 mmol Citrate solution pH 6.0 in an immunohistochemistry grade pressure cooker (e.g., Biocare Medical, Walnut Creek, CA) for 10 minutes at 110 C. Wash in running tap water for 1-2 minutes in coplin jar. Incubate in distilled water for 3 minutes. Circle off tissue area of interest using PAP pen. Wash in PBS 2 x 2 minutes.	15 minutes
Component 1	Blocking Solution (ready to use)	Incubate sections with 1-3 drops (depending on section size) of ready to use blocking solution for 20 minutes at room temperature. Drain the solution by blotting on paper towels or other adsorbent material. DO NOT RINSE	20 minutes
Component 2	Detector Antibody – anti GPC-3. (Ready to use.)	Add two or more drops (depending on section size) and incubate at room temperature for 2 hours. For added sensitivity over night incubations may be used. Wash slides with PBS 2 x 2.5 minutes.	120 minutes – 12-18 hours
Component 3	HRP Conjugate Reagent (ready to use).	Add ready to use HRP reagent to each slides, 2-3 drops depending on section size. Incubate 30 minutes at room temperature. Wash slides for five (5) minutes in wash buffer (PBS).	30 minutes
Component 4A and 4B	Substrate Reaction Buffer	Add 1 ul DAB concentrate for every 29 ul Substrate Reaction Buffer (assume	15 minutes

	and DAB concentrate	approximately 100 ul/slide). For 10 slides, one (1) drop DAB concentrate to 1 ml of Substrate Reaction Buffer. Mix well and add two or more drops per slide and incubate for 10 minutes. Wash with distilled water for two minutes.	
Component 5	Hematoxylin Concentrate	Add two or drops of hematoxylin per slide and incubate at room temperature for 1-5 minutes. Wash slides briefly with tap water. Incubate slides for 5 minutes in PBS until color turns blue. Wash with distilled water for two minutes.	10 minutes
Component 6	Mounting Media	Dehydrate slides. Incubate slides in 90% ethanol for 30 seconds followed by 30 seconds each (times 2 each) in 100 % ethanol and finally xylene. Add 1-2 drops mounting media and add coverslip.	15 minutes

* Hydrogen peroxide is not stable for long periods of time. Be sure the reagent you are using has not expired.

Sample Pictures

Example picture of formalin-fixed, paraffin embedded tissue section stained using BioMosaics GPC-3 Immunohistochemistry kit (Hepatocellular Carcinoma on cirrhotic liver tissue background).

