



Instruction for Use

【Product name】

Glypican-3 Antibody (Immunohistochemistry)

【Packing specification】

0.2ml, 1ml, 3ml, 6ml, 11ml

【Intended use】

This reagent is used for immunohistochemical staining based on routine staining to provide auxiliary information for physicians in diagnosis.

【Principle】

Glypican-3 (GPC3) is a glycosylphosphatidyl inositol-anchored membrane protein, which may also be found in a secreted form. GPC3 belongs to a member of the glypican family of heparan sulfate proteoglycans. This protein may be involved in the suppression /modulation of growth in the predominantly mesodermal tissues and organs. Glypican-3 is thought to regulate tissue and organ growth through interactions with growth factors such as Insulin-like growth factor II or fibroblast growth factor 2. GPC3 was identified to be useful tumor marker for the diagnosis of hepatocellular carcinoma (HCC), hepatoblastoma, melanoma, and testicular germ cell tumors. A high level of GPC3 expression has also been found in some types of embryonic tumors, such as Wilms tumor and hepatoblastoma. Add the primary antibody to bind the antigen on tissue sections, and then use HRP labeled secondary antibody binding primary antibody to form the secondary antibody-primary antibody-antigen complex. When DAB chromogenic solution is added, HRP reacts with enzyme substrate to produce brown insoluble reaction product, which indirectly indicating the existence of antigen.

【Main components】

Immunoglobulin, antibody diluent

【Storage】

Store at 2~8°C for 18 months.

【Sample requirements】

FFPE tissues are usually cut into sections as thin as 3~5μm with a microtome. These sections are then mounted onto glass slides that are coated with a tissue adhesive.

【Protocol】

1. Sample preparation: Deparaffinize the slides in xylene I, II, III for 5 minutes separately; Transfer the slides once through 100%, 100%, 95%, 75% alcohols for 2 minutes respectively. Rinse slides with deionized water for 30 seconds.
2. Blocking: Block endogenous peroxidase activity by incubating sections in 3% H₂O₂ solution at room temperature for 5 minutes. Rinse the slides with deionized water for 30 seconds.
3. Antigen retrieval: Heat the EDTA Antigen retrieval buffer to 100°C. Then place the slides in the boiled buffer for 15~20 min. Naturally cool down for 30 minutes at room temperature (time can be extended if needed) . Rinse the slides with washing buffer.
4. Soak slides in the washing buffer for 2 times, 3 minutes for each time. Shake off washing buffer from slides, and remove residual washing buffer around the tissue by absorbent paper, then use PAP pen to circle the tissue.
5. Primary antibody incubation: Add primary antibody to tissue, incubate at room temperature for 30 minutes. Rinse the slides in washing buffer for 3 times, 3 minutes for each time. If the antibody is concentrated, please dilute it to RTU(ready to use) according to the information on packing.
6. Secondary antibody: Drain the slides. Add secondary antibody to tissue and incubate at room temperature for 20 minutes. Rinse the slides in washing buffer for 3 times, 3 minutes for each time.
7. DAB: Drain the slides. Add DAB to the tissue and incubate at room temperature for 5 min. Rinse slides with deionized water.
8. Hematoxylin staining: Drain the slides. Add Hematoxylin to the tissue and incubate at room temperature for 5 minutes. Rinse slides with water. Use the acid solution for differentiation. Rinse slides with water.
9. Dehydrate: Dehydrate the slides in 75%, 95%, 100% alcohols for 2 minutes separately. Dry the slides and cover them with coverslips by mounting medium.

【Positive localization】

1. Positive localization: cytoplasm.
2. Positive control: hepatocellular carcinoma.

【Precautions】

1. Please read the instruction carefully and become familiar with all components of the kit prior to use, Strictly follow the instruction during operation.
2. DO NOT use the kit or any kit component after their.
3. Only trained professionals can use this kit. Please wear suitable lab coat and disposable gloves while handling the reagents.
4. Avoid contact of skin, eyes and mucous membranes with the chemicals.
5. DO NOT pipet by mouth.
6. Unused reagents, used kit and waste must be disposed according to local regulations.



【Labels, Packing Logo Design】

Symbol	Introductions	Symbol	Introductions
	Batch Code		Catalogue number
	Warnings and Precautions		non-sterile
	In Vitro diagnostic Medical device		Manufacture Date
	Manufacturer Name Address		Name and Address of European Union Representative
	CE Symbol		Unique Device Identification
	Country of Manufacture		Model Number
	Importer		Distributor
	Do not reuse" are "single use, "Use only once		

【Manufacturer】



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