

GAPDH (Glyceraldehyde-3-phosphate dehydrogenase)

GAPDH (Glyceraldehyde-3-phosphate dehydrogenase) is well-known as one of the key enzymes involved in glycolysis. It catalyzes the reversible oxidative phosphorylation of glyceraldehyde-3-phosphate. Besides functioning as a glycolytic enzyme in cytoplasm, recent evidence suggest that mammalian GAPDH is also involved in a great number of intracellular processes such as membrane fusion, microtubule bundling, phosphotransferase activity, nuclear RNA export, DNA replication and repair.

During the last decades many findings have been made concerning the role of GAPDH in different pathologies including prostate cancer progression, programmed neuronal cell death and age-related neuronal diseases such as Alzheimer's and Huntington's diseases.

GAPDH molecule is a homotetramer composed of 36 kDa subunits. Thus the molecular weight for the whole molecule is 144 kDa. Since it is constitutively and stably expressed in almost all tissues at high level, GAPDH became a well-established "housekeeping" protein and is widely used as a loading control for protein normalization in such procedures as Western blotting. It is also useful for cell visualizing in microscopy assays. Some physiological factors, such as hypoxia and diabetes, can increase GAPDH expression in certain cell types.

MONOCLONAL ANTIBODIES SPECIFIC TO GAPDH

Anti-GAPDH monoclonal antibodies developed by Hytest, especially the well characterized MAb 6C5cc, are suitable for GAPDH immunodetection in Western blotting, sandwich immunoassays and immunocytochemical applications.

Sandwich immunoassay for quantitative GAPDH immunodetection

The GAPDH-specific MABs were tested in pairs as capture and detection antibodies to select the best two-site MAB combination for the development of quantitative sandwich immunoassay.

The best selected pair for quantitative human GAPDH immunodetection is (capture-detection): 6C5cc - 4G5.

GAPDH immunodetection in direct ELISA

All anti-GAPDH MABs recognize human GAPDH in direct ELISA (Fig. 1).

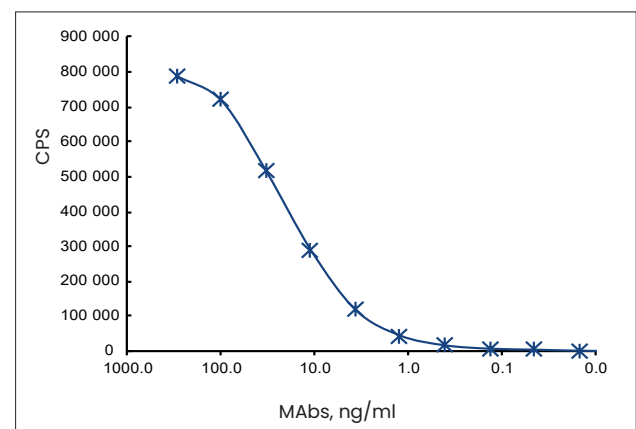


Figure 1.
Interaction of anti-GAPDH MAb 4G5 with GAPDH, isolated from human heart tissue in direct fluorimmunoassay. 100 ng of human antigen per well for plate coating was used.

GAPDH immunodetection in Western blotting

Anti-GAPDH MAbs were tested on their ability to recognize GAPDH from tissue extracts of different animal species and lysates of different cell types after Western blotting (Fig. 2). All MAbs detect human GAPDH in Western blotting with high sensitivity and demonstrate cross-reactivity with GAPDH from wide range of species. It makes possible their application in biochemical and immunochemical studies of very diversified objects.

We strongly recommend MAb 6C5cc as the most suitable for Western blotting, but MAb 4G5 can be used successfully in Western blotting studies also. Recommended MAb concentration for Western blotting is 0.5-1 µg/ml. Standard protocols for Western blotting can be easily applied to all anti-GAPDH MAbs, as was confirmed in numerous investigations (see references on page 3).

Anti-GAPDH MAbs produced by Hytest have wide species cross-reactivity and could be chosen in accordance with the customers needs (see Table 1). Since GAPDH is highly conserved across different species, it is probable, that Hytest anti-GAPDH MAbs can be applied for much more species not tested yet.

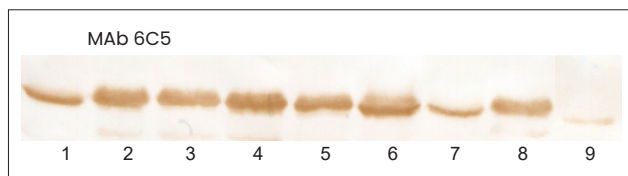


Figure 2. Immunodetection of GAPDH in tissue extracts using MAb 6C5 in Western blotting after SDS-PAGE in reducing conditions. Heart extracts were prepared from different animal species. About 0.25 mg of homogenized wet cardiac tissue per track was loaded. MAb 6C5 was conjugated with HRP.

Track 1: human, Track 2: canine, Track 3: feline,
Track 4: porcine, Track 5: rabbit, Track 6: rat,
Track 7: duck, Track 8: chicken, Track 9: fish

Table 1. MAbs cross-reaction with GAPDH from different animal species.

MAb	Cross-reaction in Western blotting									
	Human	Bovine	Porcine	Goat	Canine	Rabbit	Cat	Rat	Mouse	Fish
6C5	+++	-	+++	-	+++	+++	+++	+++	+++	+++
4G5	++	++	++	++	+	+	++	++	++	+

GAPDH immunoprecipitation from tissue extracts and cell lysates

Immunoprecipitation is a widely used procedure by which peptides or proteins that react specifically with an antibody could be removed from the solution. This technique provides a rapid and simple method to separate a specific protein from whole cell lysates, tissue extracts or culture supernatants. Additionally, the method could be used to study biochemical characteristics, post-translational modifications, expression levels or to confirm identity of the protein of interest. Some of Anti-GAPDH MAbs offered by Hytest could be applied for immunoprecipitation. Fig. 3 represents the examples of MAbs 6C5 and 4G5 application for GAPDH extraction from different tissue extracts.

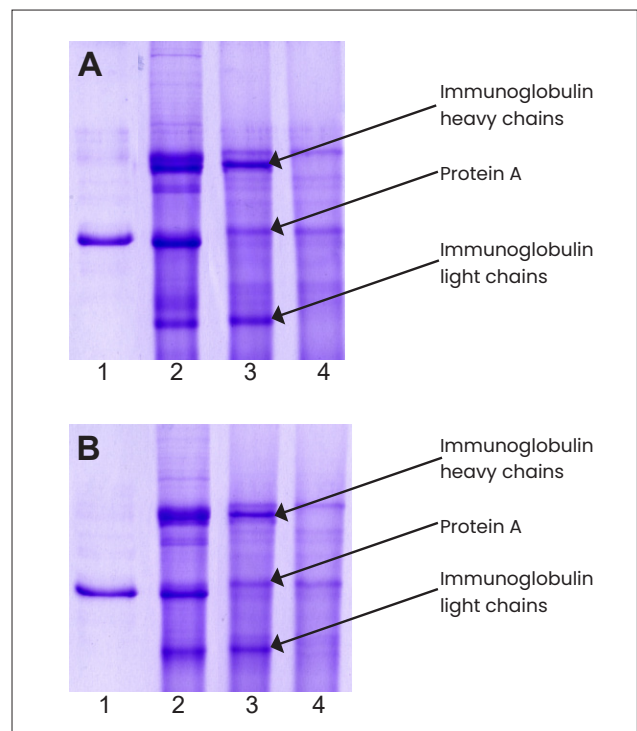


Figure 3. Immunoprecipitation of GAPDH from rat heart extract using anti-GAPDH MAb 6C5 (A) or MAb 4G5 (B).

Mixture of protein A-Sepharose with anti-GAPDH MAbs and tissue extract was incubated for 30 min at room temperature and precipitated by centrifugation. Pellet was washed with PBS, suspended in reducing electrophoresis sample buffer and heated for 5 minutes at 100 °C. After centrifugation supernatant was loaded on gel and proteins were separated by SDS electrophoresis.

Track 1: Human GAPDH (1 µg)
Track 2: GAPDH immunoprecipitated from rat heart tissue extract
Track 3: Only MAb 6C5 (A) or 4G5 (B) preincubated with Protein A Sepharose
Track 4: Only Protein A Sepharose

Immunocytochemical detection of GAPDH

In living cell GAPDH is represented mainly in cytoplasm. Recent investigations revealed that depending on cell metabolic status GAPDH can be associated with other cellular compartments, such as lysosomes, synaptic vesicles, cytoskeleton and plasma membranes. As a soluble protein, GAPDH was shown to serve as a transporting protein between intracellular sites. GAPDH translocation to the nucleus is considered to be in association with cell death. It is known that nuclear form of GAPDH differs in conformation and biochemical properties from cytoplasmic one and is colocalized with fragmented and/or condensed chromatin. Immunocytochemical detection of exact GAPDH localization is in such a way extremely useful and approved tool in investigation of cell's metabolism and functional activity. Fig. 4 represent Hytest anti-GAPDH MAbs application in immunocytochemical detection of GAPDH in different cell types.

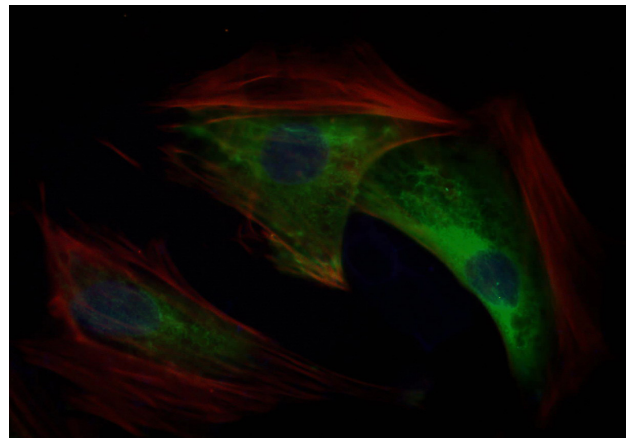


Figure 4.
Immunostaining of GAPDH in A-10 cell line (rat aortic smooth muscle cells).

Cells were fixed by formalin and GAPDH was stained by:
MAb 6C5 (green colour)
F-actin microfilaments-binding dye (red)
DNA-binding dye (dark blue)

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ORDERING INFORMATION

MONOCLONAL ANTIBODIES

Product name	Cat. #	MAB	Subclass	Remarks
Glyceraldehyde 3-phosphate dehydrogenase (GAPDH)	5G4	4G5	IgG1	EIA, WB, IF, IHC, IP
	5G4cc	6C5cc	IgG1	<i>In vitro</i> , EIA, WB, IF, IHC, IP, C/r data available (WB control)

Please note that some or all data presented in this TechNotes has been prepared using MAbs produced *in vivo*. MAbs produced *in vitro* are expected to have similar performance.