HyTest NEWS

GAPDH Newsletter

General

GAPDH (Glyceraldehyde-3-phosphate dehydrogenase) is well-known as one of the key enzymes involved in glycolysis. It catalyzes the reversible oxidative phosphorylation of glyceraldehydes-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.

Anti-GAPDH monoclonal antibodies developed by HyTest, especially the well characterized MAb 6C5, are suitable for GAPDH immunodetection in Western blotting, sandwich immunoassays and immunocytochemical applications.

1. GAPDH Antigen

Source: Cardiac tissue
Purity: >98% by SDS-PAGE
Presentation: Suspension in 80% ammonium sulphate with traces of HEPES, EDTA and DTT.
Application: Standard or calibrator in immunoassays, immunogen for antibody production, biochemcal and immunochemical studies

GAPDH is purified from human or rabbit cardiac tissue and can be used as a standard or calibrator in immunoassays, as an immunogen for antibody production, and in GAPDH biochemical and immunochemical studies. On gel electrophoresis under reducing conditions and on Western blots GAPDH is detected as a single band with apparent molecular mass of about 36 kDa (Fig. 1).

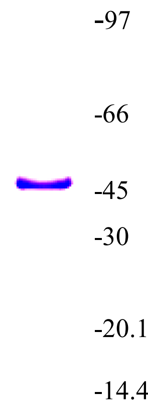

Figure 1. SDS-PAGE of human GAPDH under reducing conditions. 0.5 µg of human GAPDH loaded on track. Positions of molecular weight standards are marked by digits on the right side of the picture

Ordering information:

Product	Cat.#	Purity	Source
GAPDH, Human	8G4	>98%	Human cardiac tissue
GAPDH, Rabbit	8G4r	>98%	Rabbit muscle tissue

2. Anti- GAPDH monoclonal antibodies

Host animal: Mice Balb/c
Cell line used for fusion: Sp2/0
Antigen: Rabbit or human GAPDH
Purification method: Chromatography on protein A Sepharose
Presentation: PBS, 0.1 % sodium azide
Applications: GAPDH immunoassays, Western blotting, immunocytochemistry and others.

Hybridomas producing MAbs were generated after Balb/c mice immunization with human or rabbit GAPDH. MAbs can be used for immunochemical detection of GAPDH in Western blotting, sandwich immunoassays, immunofluorescence, immunocytochemistry and other applications.

2.1. 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 6C5 as the most suitable for Western blotting, but other MAbs can be used successfully in Western blotting studies also. Recommended MAb concentration for Western blotting is 0.5-1μg/ml. Standard protocols for Western blotting can be easily applied to all anti-GAPDH MAbs, as was confirmed in numerous investigations (see references below).

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.

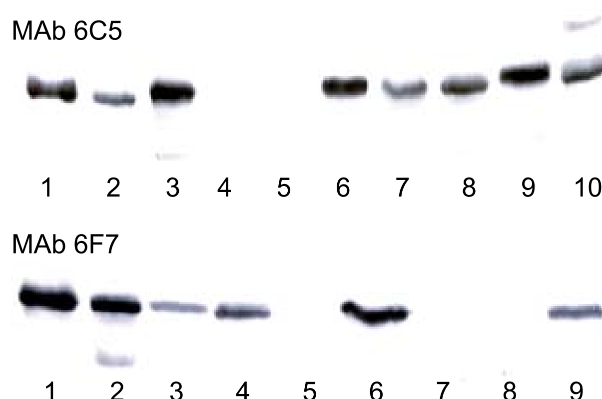

Figure 2. Immunodetection of GAPDH in tissue extracts using monoclonal antibodies in Western blotting after SDS-PAGE in reducing conditions.
MAb 6C5 in upper picture, MAb 6F7 in lower picture. Heart extracts were prepared from different animal species. About 0.5 mg of homogenized wet cardiac tissue per track was loaded. Anti-mouse IgG conjugated with HRP was used for MAb-GAPDH immune complex visualization.

Track 1: isolated human GAPDH, 0.5 μg
Track 2: human heart tissue extract
Track 3: pig heart tissue extract
Track 4: goat heart tissue extract
Track 5: bovine heart tissue extract
Track 6: dog heart tissue extract
Track 7: mouse heart tissue extract
Track 8: rat heart tissue extract
Track 9: rabbit heart tissue extract
Track 10: duck heart tissue extract

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

	Cross-reaction in Western blotting									
MAb	Human	Bovine	Porcine	Goat	Canine	Rabbit	Cat	Rat	Mouse	Fish
6C5	+++	-	+++	-	+++	+++	+++	+++	+++	+++
6F7	++	-	+++	+++	+++	+++	+++	-	-	-
9B3	++	++	+	+	-	+	-	+	-	-
10B8	++	++	+	+	-	+	-	+	-	-
4G5	++	++	++	++	+	+	++	++	++	+

2.2. GAPDH immunodetection in direct ELISA

All anti-GAPDH MAbs recognize human GAPDH in direct ELISA (Fig. 3).

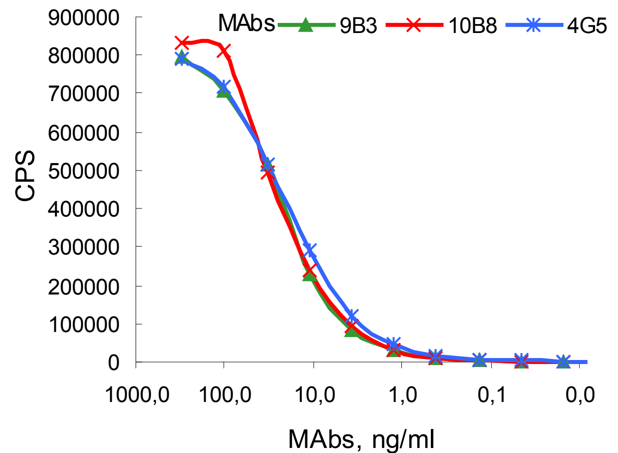

Figure 3. Interaction of anti-GAPDH antibodies 9B3, 10B8 and 4G5 with GAPDH, isolated from human heart tissue in direct fluoroimmunoassay.
100 ng of human antigen per well for plate coating was used.

2.3. Sandwich immunoassay for quantitative GAPDH immunodetection

All HyTest's 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.

Calibration curve for the immunoassay, utilizing MAb 6F7 for capture and MAb 4G5 for detection (labelled with stable Eu^{3+} chelate) is shown on Fig. 4.

The best selected MAb combinations for quantitative human GAPDH immunodetection are (capture-detection respectively):

6C5 - 4G5
6C5 - 9B3
6F7 - 9B3
6F7 - 4G5

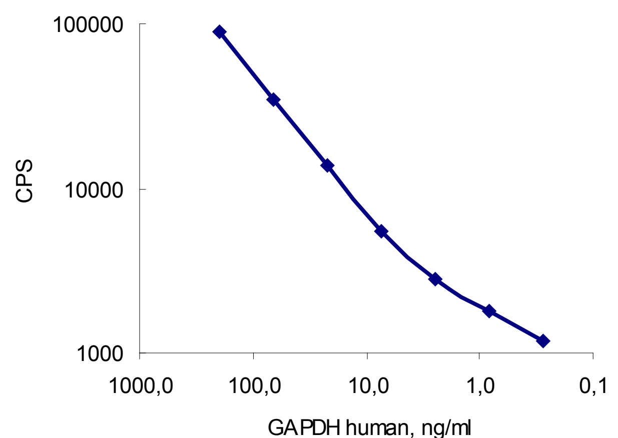

Figure 4. Calibration curve for GAPDH sandwich immunoassay.
Antigen: Human GAPDH
Capture MAb: 6F7 served as a coating,
Detection MAb: 4G5 (labelled with stable Eu^{3+} chelate).

2.4. 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 lysozymes, 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. 5 and Fig. 6 represent HyTest anti-GAPDH MAbs application in immunocytochemical detection of GAPDH in different cell types.

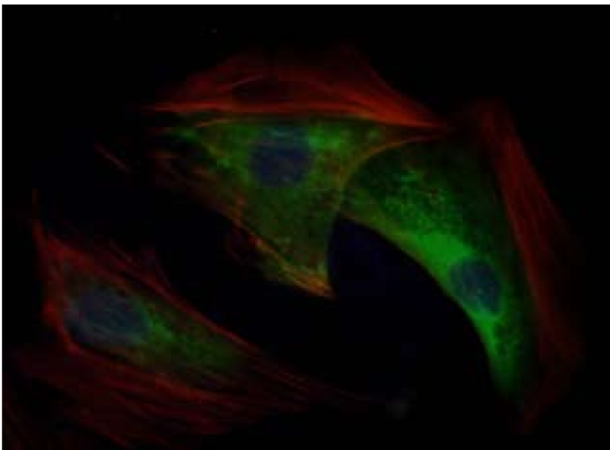

Figure 5. Immunostaining of GAPDH in A-10 cell line (rat aortic smooth muscle cells).
Cells were fixed by formalin and GAPDH was stained by:
- MAb 6C5 (green colour)
- F-actin microfilaments-binding dye (red)
- DNA-binding dye (dark blue)

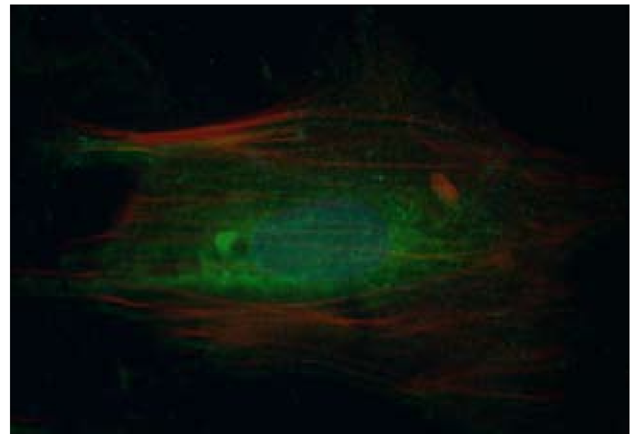

Figure 6. Immunostaining of GAPDH in human marrow stromal cells.
Cells were fixed by formalin and GAPDH was stained by:
- MAb 9B3 (green colour)
- F-actin microfilaments-binding dye (red)
- DNA-binding dye (dark blue)

2.5. 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. 7 represents the examples of MAbs 6C5 and 4G5 application for GAPDH extraction from different tissue extracts.

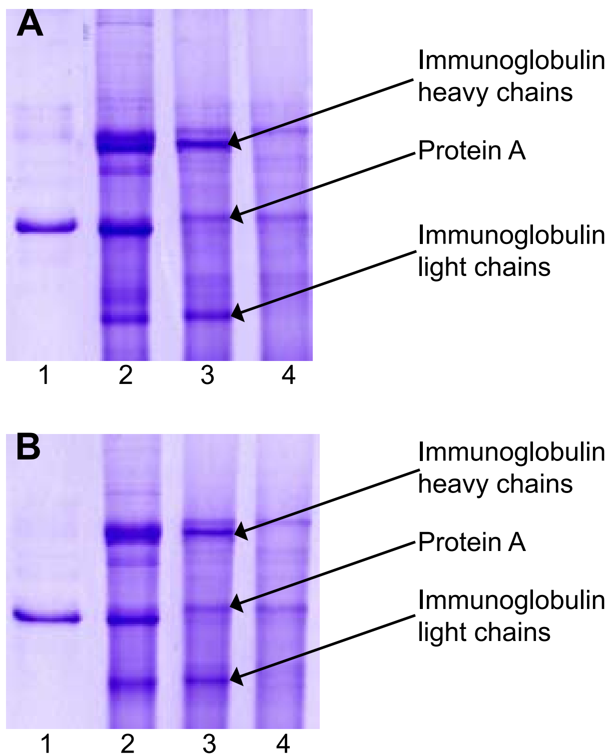

Figure 7. Immunoprecipitation of GAPDH from rat heart extract using anti-GAPDH MAb 6C5 (A) or MAb 4G5 (B).
Mixture of protein A-Sepharose with anti-GAPDH MAbs and tissue extract was incubated for 30 min at room temperature and precipitated by centrifugation. Pellet was washed with PBS, suspended in reducing electrophoresis sample buffer and heated for 5 minutes at 100 ºC. 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

Ordering information:

Product	Cat.#	MAb	Subclass	Application
Anti-GAPDH	5G4	6C5	IgG1	EIA, WB, IF, IC, IP
Anti-GAPDH	5G4	6F7	IgG1	EIA, WB, IF, IC
Anti-GAPDH	5G4	10B8	IgG1	EIA, WB, IF, IC
Anti-GAPDH	5G4	4G5	IgG1	EIA, WB, IF, IC, IP
Anti-GAPDH	5G4	9B3	IgG1	EIA, WB, IF, IC

For Western blotting application we can provide a combination of selected antibodies (depending on animal species specificity) and GAPDH antigen sample with known concentration ready to use as a control for SDS gel electrophoresis after reconstitution in water. Please do not hesitate to ask for additional information.

2.4. References

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9. Dainiak MB, Izumrudov VA, Muronetz VI, Galaev IY, Mattiasson B. Conjugates of monoclonal antibodies with polyelectrolyte complexes-- an attempt to make an artificial chaperone. Biochim Biophys Acta. 1381(3):279-85 (1998).

Intelligate, Joukahaisenkatu 6
20520 Turku, FINLAND
Tel. +358-2-512 0900 Fax +358-2-512 0909
E-mail: hytest@hytest.fi
Internet: www.hytest.fi

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