

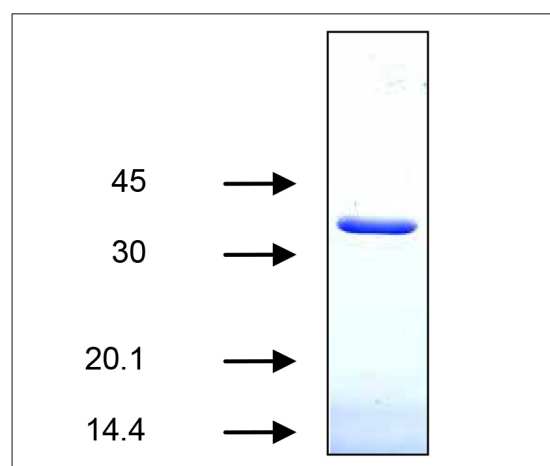
## GAPDH (Glyceraldehyde-3-phosphate dehydrogenase)



### GAPDH antigen

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. 2 µg of human GAPDH loaded on track. Positions of molecular weight standards are marked on the left side of the picture*

### 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.

### Applications

#### GAPDH immunodetection in Western blotting

Anti-GAPDH MAbs were tested for 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. **MAb 6C5 is recommended as the most suitable for Western blotting, but other MAbs can be**

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.

#### used successfully in Western blotting.

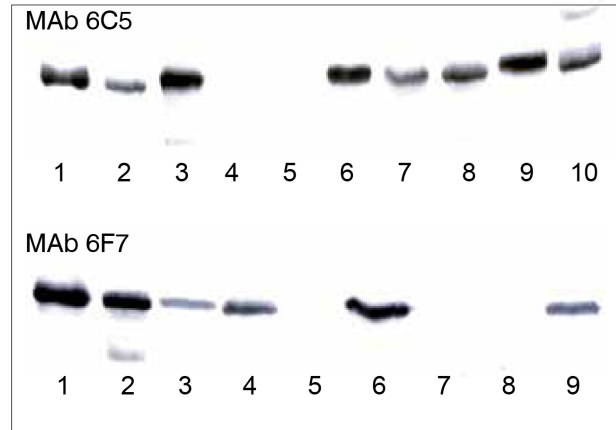
Recommended MAb concentration is 0.5-1 µg/ml. Standard protocols for Western blotting can be easily applied to all anti-GAPDH MAbs, as was confirmed in numerous investigations (see references on last page).

Anti-GAPDH MAbs have wide species cross-reactivity and can be chosen in accordance with the customers needs (see Table 1). Since GAPDH is highly conserved across different species, it is probable, that 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, 0.5µg
- 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



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

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

**GAPDH immunodetection in direct ELISA**

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

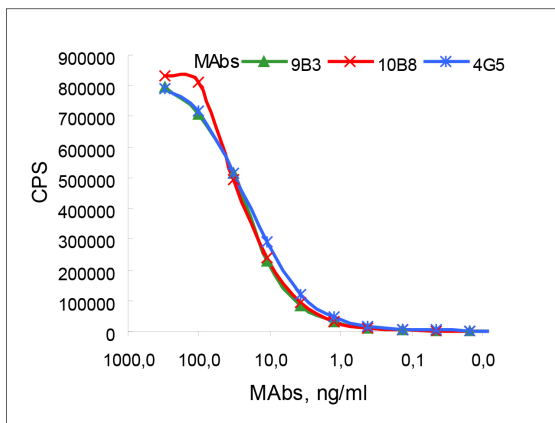


Figure 3. Interaction of anti-GAPDH antibodies 9B3, 10B8 and 4G5 with GAPDH, isolated from human heart tissue in direct fluoroimmunoassay. 100 ng of human antigen per well for plate coating was used.

**Sandwich immunoassay for quantitative GAPDH immunodetection**

All 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<sup>3+</sup> chelate) is shown on Fig. 4.

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

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

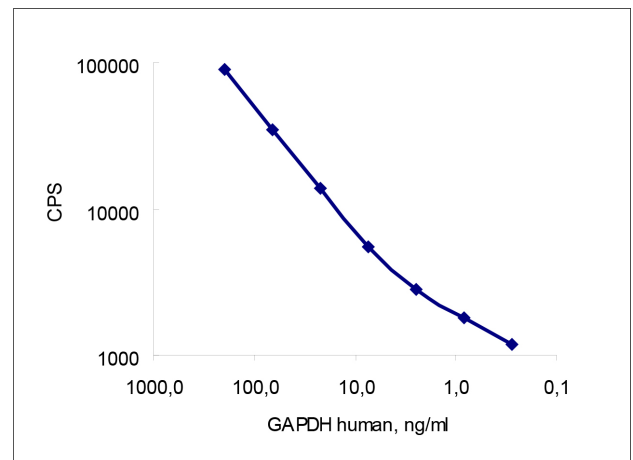


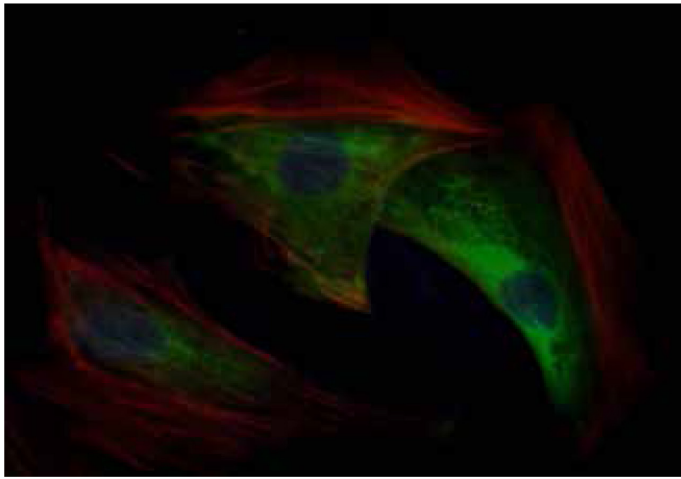
Figure 4. Calibration curve for GAPDH sandwich immunoassay.

Antigen: Human GAPDH  
 Capture MAb: 6F7  
 Detection MAb: 4G5 (labelled with stable Eu<sup>3+</sup> chelate)

**Immunocytochemical detection of GAPDH**

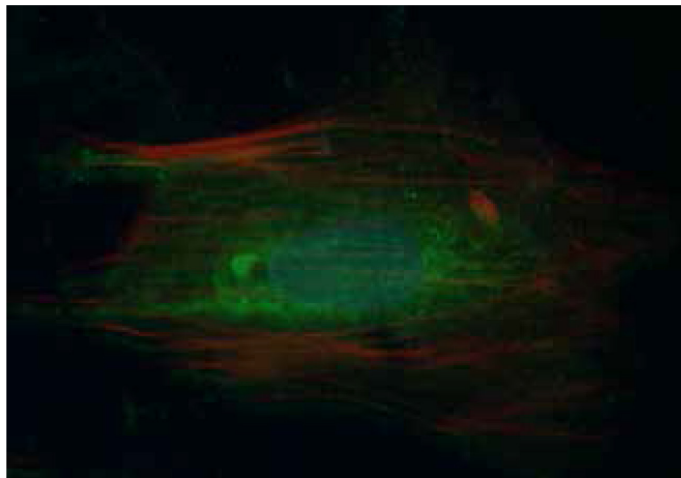
In living cell GAPDH is represented mainly in cytoplasm. Recent investigations revealed that depending on cell metabolic status GAPDH can be associated with other cellular compartments, such as lysozymes, synaptic vesicles, cytoskeleton and plasma membranes.

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 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)

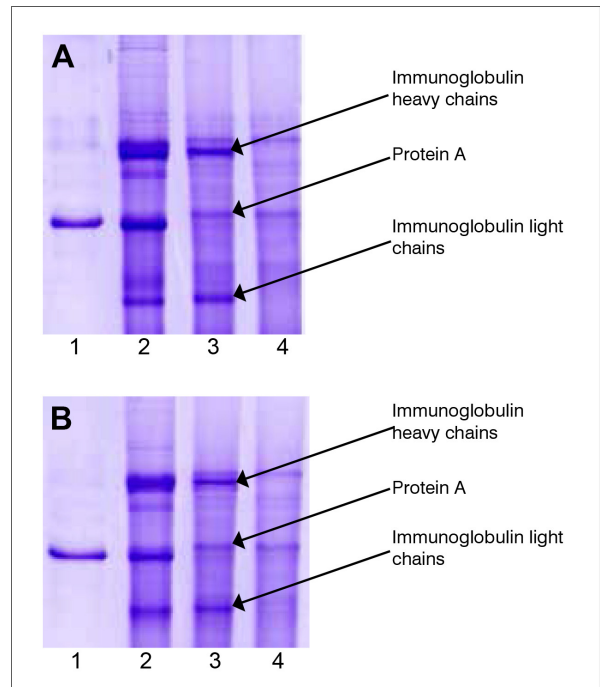


**Figure 6. Immunostaining of GAPDH in human marrow stromal cells.**

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)

## 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 Advanced ImmunoChemical can 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:**  
**MONOCLONAL ANTIBODIES**

Product Name	Cat #	MAb	Subclass	Remarks
GAPDH	2-RGM2	6C5	IgG1	EIA, WB, IF, IHC, IP, C/r data available (WB control)
		10B8	IgG1	EIA, WB, IF, IHC
		4G5	IgG1	EIA, WB, IF, IHC, IP
		6F7	IgG1	EIA, WB, IF, IHC
		9B3	IgG1	EIA, WB, IF, IHC

**ANTIGEN**

Product Name	Cat #	Purity	Source
GAPDH, human	8-GAPDH-h	>98%	Human cardiac tissue
GAPDH, human	8-GAPDH-r	>98%	Rabbit cardiac tissue

**References**

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