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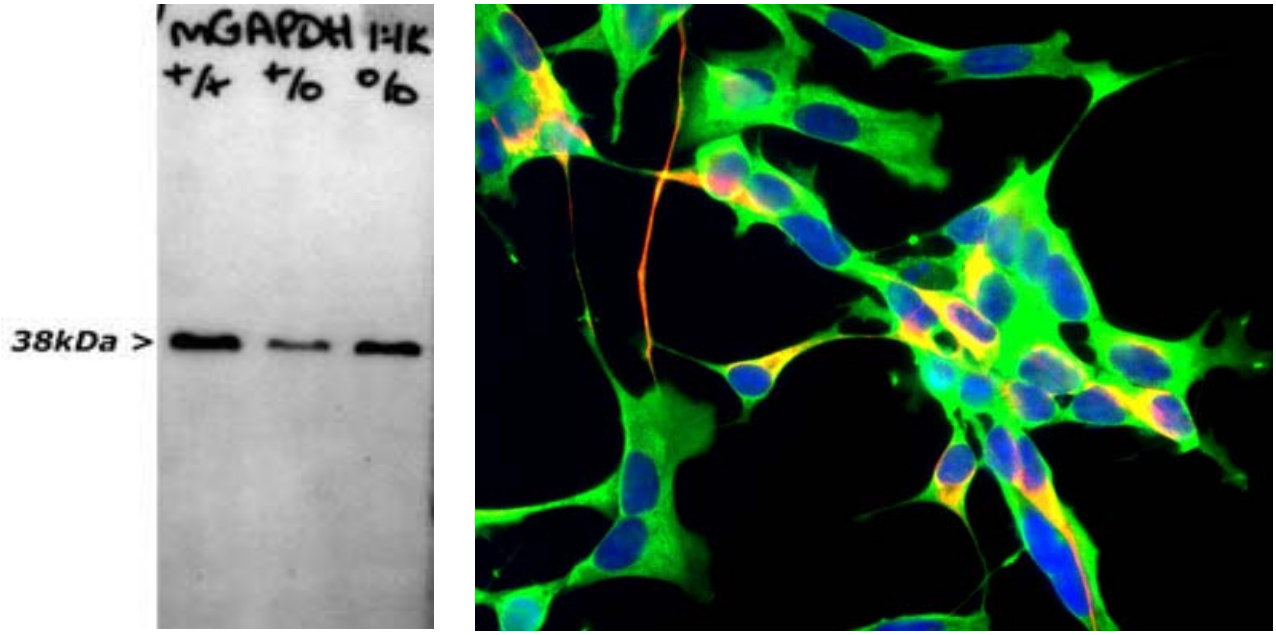
Catalogue# MCA-1D4: Mouse Monoclonal Antibody to Glyceraldehyde 3-Phosphate Dehydrogenase (GAPDH, G3PDH or GPDH)

The Immunogen: Glyceraldehyde 3-Phosphate Dehydrogenase (GAPDH) is a metabolic enzyme responsible for catalyzing one step in the glycolytic pathway, the reversible oxidative phosphorylation of glyceraldehyde 3-phosphate. Because GAPDH as a protein expressed in large amounts and which is required at all times for important "house keeping" functions, levels of GAPDH mRNA are often measured and used as standards in studies of mRNA expression. Increasingly, scientists are making use of specific antibodies to GAPDH in comparable studies of levels of protein expression. This antibody can be used as a loading control for western blotting experiments, allowing comparison between the level of this protein and others in a cell or tissue. Apart from a role in glycolysis, GAPDH may have other roles such as in the activation of transcription (1). GAPDH is reported to bind to a variety of other proteins, including the amyloid precursor protein, mutations in which cause some forms of Alzheimer's disease, and the polyglutamine tracts of Huntingtin, the protein product aberrant forms of which are causative of Huntington's disease (2,3). Associations with actin and tubulin have also been reported. The protein may also have a role in the regulation of apoptosis, and interestingly migrates from the cytoplasm into the nucleus when cells become apoptotic (4). The immunogen used to raise this particular antibody was extensively purified pig GAPDH. This antibody has been widely used and is marketed by several other companies besides EnCor. However we made it and we can sell it for less than anyone else. References 5 and onwards made use of this antibody in a peer reviewed publication.



Left: blots of crude extract of peripheral nerve of various knock out mice strains blotted with MCA-1D4 for use as a western blotting control. Note the single sharp clean band at 38 kDa corresponding to GAPDH. **Right:** Human neuroblastoma SH-SY5Y cells stained with MCA-1D4 (green), chicken antibody to neurofilament NF-H CPCA-NF-H (red) and DNA (blue). The antibody reveals strong cytoplasmic staining for GAPDH. Some of the cells are also rich in NF-H.

Antibody characteristics: MCA-1D4 is a mouse IgM class antibody with a κ light chain. IgM antibodies differ from IgGs in that they contain a total of ten antigen binding sites instead of only two. They can be used in the same was as IgGs, and most secondary reagents binding IgGs will also bind IgMs. MCA-1D4 recognizes GAPDH specifically both in western blots and in immunocytochemical experiments. On blots MCA-1D4 reveals a prominent ~38 kDa band, and on cells in tissue culture the antibody stains in a diffuse somewhat punctate cytoplasmic fashion, generally with little nuclear stain. MCA-1D4 is known to react with GAPDH from human,

cow, pig, mouse, rat and other mammals, and also recognizes avian GAPDH. Since GAPDH is one of the most conserved proteins known, it is likely that the antibody is effective on other species also.

Suggestions for use: The antibody solution was made using Integra CL-350 cells and is therefore concentrated tissue culture supernatant, with an antibody concentration of at least 1mg/ml. The antibody solution can be used at dilutions of at least 1:100 in immunofluorescence experiments. In western blotting using chemiluminescence it can be used at dilutions of 1:1,000 or lower. It is very useful as a loading control for quantitative western blotting (see for example references 5 and 6). Antibody preparation contains 10mM sodium azide preservative (Link to http://www.encorbio.com/MSDS/azide.htm for Material Safety Data Sheet). Avoid repeated freezing and thawing, store at 4°C or -20°C.

Limitations: This product is for research use only and is not approved for use in humans or in clinical diagnosis.

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