

DATASHEET

Anti-HA ATTO488

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Mouse monoclonal antibody
Anti-HA (clone 12CA5)

For Laboratory Use Only.
Not for Use in Diagnostic Processes.

Quantity: 200 µg (lyophilized)
Cat. #: 7802-01

Product Description

The HA Tag (hemagglutinin tag) is an epitope tag, commonly used to localize gene products in many types of cultured cells, to study the topology of proteins and protein complexes. Anti-HA is especially useful whenever no primary antibody against the protein of interest is available, mostly because it is less immunogenic and low abundant.

Monoclonal Anti-HA is directed against a peptide epitope of the hemagglutinin (HA) glycoprotein derived from recombinant hemagglutinin protein (aa 98-106) of the human influenza virus. The antibody is used for the immunodetection and functional characterization of native and denatured protein. It is frequently used for the detection and characterization of recombinant expressed HA-tagged proteins *in vivo* and *in vitro* or proteins containing the epitope YPYDVPDYA.

Anti-HA ATTO488 facilitates protein detection in immunofluorescence & -chemistry, western blotting etc. of tagged proteins.

Anti-HA is protein G purified from cell culture supernatant with a resulting purity of ~99% as determined by SDS-PAGE. Anti-HA ATTO488 (λ_{ex} 501nm, λ_{em} 523nm) has been chemically modified by coupling of ATTO488 NHS to the antibody, without impairing its biological activity. The antibody has an isoelectric point of 6.4 and is subtype-classified as IgG_{2b}. The affinity given by the dissociation constant is: $K_d = 1 \times 10^8 / M$.

Reconstitution of Anti-HA

Add 0.1ml Glycerol-PBS to the lyophilized antibody to reconstitute a 1mg/ml stock solution. Vortex for 10sec until completely dissolved. Final concentrations of the antibody buffer: 0.01M sodium phosphate, 0.1M NaCl, pH 7.4, 0.2mM NaN₃ in 50% glycerol.

Working Dilutions

Each individual user should determine the optimum working dilution empirically for the systems. We recommend starting with dilutions for western blotting of 1:200-1:2000, immunoprecipitations 1-2µg per 100-500µl of total protein/ml and for immunofluorescence 1-10µg/ml.

For product inquiries please contact:

cusserv@hypermol.com
Fon: +49 (0)521 9876228
Fax: +49 (0)521 9876231
www.hypermol.com

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Specifications of Anti-HA ATTO488

Esterification with ATTO488 results in a chemically modified antibody carrying a fluorescent dye with a net electrical charge of 0. The fluorescence is excited most efficiently at 523nm.

Determining the Degree of Labeling (DOL)

1. Protein Concentration

Determination of the protein concentration by UV absorption measurement at 280nm ($\epsilon_{\max} = 203,000 \text{ M}^{-1}\text{cm}^{-1}$).

2. Degree of Labelling

The degree of labeling (DOL or dye/protein ratio) is usually determined by absorption spectroscopy making use of the Lambert-Beer law: Absorbance (A) = extinction coefficient (ϵ) \times molar concentration \times path length (d). Simply measure the UV-VIS spectrum of the conjugate in solution in a quartz cuvette. Dilute the solution, if necessary to measure within the linear range.

$$\text{DOL} = \frac{A_{501} \cdot 203,000}{A_{280} - (A_{501} \cdot 0.1) \cdot 90,000}$$

A_{501} = maximal absorbance at 501nm measured in a cuvette with a pathlength of 1 cm.

A_{280} = maximal absorbance at 280nm measured in a cuvette with a pathlength of 1 cm.

203,000 = molar extinction coefficient (ϵ) at the longest-wavelength absorption maximum ($\text{M}^{-1}\text{cm}^{-1}$).

90,000 = molar extinction coefficient (ϵ) at the longest-wavelength absorption maximum ($\text{M}^{-1}\text{cm}^{-1}$).

0.1 = correction factor for the fluorophore's absorbance at 280nm.

Storage and Stability

For continuous use, store at 2-8 °C for up to three months. For extended storage, the solution may be frozen in working aliquots at -20 °C. Frozen aliquots are stable for at least six months. Avoid repeated freeze/thawing. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Protect fluorescent conjugates from light.

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