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DATASHEET

Fluorescent Antibody Kit
Atto390

gar IgG (H+L) Atto390

Goat-anti rabbit IgG (H+L) Atto390

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

Kit Content (Cat. #: 2301-1MG)

1.0mg gar IgG (H+L) Atto390
50µg mono-anti actin
Product documentation & Certificate of Analysis

Product Documentation

Goat anti-rabbit IgG (H+L) Atto390

Goat anti-rabbit IgG (H+L) is an antigen-specific antibody. Affinity purification removed essentially all goat serum proteins, including immunoglobulins not specifically binding to rabbit IgG. Goat anti-rabbit IgG is conjugated to Atto390 (Abs.max. 390 nm; Em.max. 479 nm) and further purified by gel filtration.

Goat anti-rabbit IgG (H+L) Atto390 is supplied in unit sizes of 1.0mg.

In solution: 0.5ml (2mg/ml) in 0.01M sodium phosphate, 0.1M NaCl, pH 7.4, 10mM NaN_3 in 50% glycerol (fluorescence free).

Reconstitution of Antibodies with Glycerol-PBS (for freeze-dried products only)

Add 0.5ml Glycerol-PBS to the freeze-dried secondary antibody to reconstitute a 2mg/ml stock solution. Vortex for 10sec until completely dissolved. Add 50µl Glycerol-PBS to the freeze-dried primary antibody to reconstitute a 1mg/ml stock solution. Final concentrations of the antibody buffers: 0.01M sodium phosphate, 0.1M NaCl, pH 7.4, 5mM NaN_3 in 50% glycerol.

Working Dilution

Each individual user should determine the optimum working dilution empirically for the systems. Dilutions of 1:300 – 1:1000 are sufficient for many applications.

Determining the Degree of Labeling (DOL)

1. Protein Concentration

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

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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) × molar concentration × 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.

$$DOL = \frac{A_{390} \cdot 203{,}000}{A_{280} - (A_{390} \cdot 0.08) \cdot 24{,}000}$$

A_{390}= maximal absorbance at 390nm 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 ($M^{-1}cm^{-1}$).

24,000 = molar extinction coefficient (ε) at the longest-wavelength absorption maximum ($M^{-1}cm^{-1}$).

0.08 = 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.

Mono-anti actin

Monoclonal anti actin (98% purity) recognizes skeletal and non-muscle actin isoforms. Isotype classified as an IgM, it reacts even stronger with goat-anti mouse IgG. In immunofluorescence microscopy samples are fixed with methanol to detect cytoplasmic actin, while fixation with para-formaldehyde leads to nuclear actin detection (Gonsior et al., 1999).

As immunogen for mono-anti actin a profilin-actin complex from calf thymus was used, and epitope mapping localized the following sequence (Gonsior et al.):
NVPAMYVAVLDSGVTHNVPIYHAIMRLDLA.

Mono-anti actin was tested on PtK2, SR-NRK, NRK-49F, L6 cells, C2C12, NIH-3T3, rabbit myoblast and myotube cells.

The antibody is supplied in unit sizes of 50µg, either in solution or freeze dried. In solution: 50µl (1mg/ml) in 0.1M sodium phosphate, 0.1M NaCl, pH 7.4, 5mM NaN_3 in 50% glycerol (fluorescence free).

Freeze dried products are reconstituted with 50µl glycerol buffer provided with the kit.

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Mono-anti actin

Working Dilution

Each individual user should determine the optimum working dilution empirically for the systems. Dilutions of 1:100 – 1:300 with respect to the above mentioned fixation methods are sufficient for many applications.

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.

Reference:
Gonsior SM, et al.: Conformational difference between nuclear and cytoplasmic actin as detected by a monoclonal antibody. J Cell Sci 112, 797-809 (1999)

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