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DATASHEET

Fluorescent Antibody Kit Atto647N

gam IgG (H+L) Atto647N

Goat-anti mouse IgG (H+L) Atto647N

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

Kit Content (Cat. #: 2108-1MG)

1.0mg gam IgG (H+L) Atto647N
50µg mono-anti actin
Product documentation & Certificate of Analysis

Product Documentation

Goat anti-mouse IgG (H+L) Atto647N

Goat anti-mouse IgG (H+L) is an antigen-specific antibody. Affinity purification removed essentially all goat serum proteins, including immunoglobulins not specifically binding to mouse IgG. Goat anti-mouse IgG is conjugated to Atto647N NHS (Abs.max. 645 nm; Em.max. 669 nm) and further purified by gel filtration.

Goat anti-mouse IgG (H+L) Atto647N 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).

Freeze dried products are reconstituted with 0.5ml glycerol buffer provided with the kit.

Working Dilution

Each individual user should determine the optimum working dilution empirically for the systems. Dilutions of 1:500 – 1:1500 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.

$$DOL = \frac{A_{644} \cdot 203{,}000}{A_{280} - (A_{644} \cdot 0.05) \cdot 150{,}000}$$

A_{644} = maximal absorbance at 644nm 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 ($M^{-1}cm^{-1}$).

150,000 = molar extinction coefficient (ε) at the longest-wavelength absorption maximum ($M^{-1}cm^{-1}$).

0.05 = 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. Although isotype-classified as IgM, it reacts even to 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 profiling-actin complex from calf thymus was used, and the epitope is located within the following sequences (Gonsior et al.):

AMYVAIQAV (aa131-139), VLDSGVTHNVPIYEGY (aa155-169) MRLDLAGRDLTD (aa178-187).

Mono-anti actin was tested on PtK2, SR-NRK, NRK-49F, L6 cells, C2C12, NIH-3T3, mouse 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 0.01M 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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