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# DATASHEET

Fluorescent Antibody Kit
ATTO488

## Anti-Rabbit IgG (H+L) ATTO488

Goat Anti-Rabbit IgG (H+L) ATTO488

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

## Kit Content (Cat. #: 2302-1MG)

1.0mg Anti-Rabbit IgG (H+L) ATTO488
1.0ml Glycerol-PBS
20µg Mono anti-actin
Product documentation & Certificate of Analysis

## Product Documentation

### Anti-Rabbit IgG (H+L) ATTO488

Anti-Rabbit IgG (H+L) is an antigen-specific antibody. Affinity purification removed essentially all goat serum proteins, including immunoglobulins not specifically binding to mouse IgG. Anti-Rabbit IgG is conjugated to ATTO488 NHS (Abs.max. 501 nm; Em.max. 523 nm) and further purified by gel filtration.

Anti-Rabbit IgG (H+L) ATTO488 is supplied in unit sizes of 1.0mg.

## Reconstitution of Antibodies with Glycerol-PBS

Add 0.5ml Glycerol-PBS to the lyophilized antibody to reconstitute a 2mg/ml stock solution. Vortex for 10sec until completely dissolved. Add 50µl Glycerol-PBS to the lyophilized 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 1:500 – 1:1500 are suitable for many applications.

## Determining the Degree of Labeling (DOL)

1. Protein Concentration

Determination of the protein concentration by UV absorption measurement at 280nm ( $\varepsilon_{max}$ =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.

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

$A_{501}$ = 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 ($M^{-1}cm^{-1}$).

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

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.

## 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 Anti-Rabbit 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.

## 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.

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Mono anti-actin

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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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