



## DATASHEET

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### Fluorescent Antibody Kit ATTO594

#### Anti-Rabbit IgG (H+L) ATTO594

Goat Anti-Rabbit IgG (H+L) ATTO594

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

#### Kit Content (Cat. #: 2306-1MG)

1.0mg Anti-Rabbit IgG (H+L) ATTO594  
1.0ml Glycerol-PBS  
50µg Mono-anti actin  
Product documentation & Certificate of Analysis

#### Product Documentation

##### Anti-Rabbit IgG (H+L) ATTO594

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 ATTO594 NHS (Abs.max. 601 nm; Em.max. 627 nm) and further purified by gel filtration.

Goat Anti-Rabbit IgG (H+L) ATTO594 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<sub>3</sub> 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 sufficient for many applications.

#### Determining the Degree of Labeling (DOL)

##### 1. Protein Concentration

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

## DATASHEET

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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.

$$\text{DOL} = \frac{A_{601} \cdot 203,000}{A_{280} - (A_{601} \cdot 0.51) \cdot 120,000}$$

$A_{601}$  = maximal absorbance at 601nm 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 ( $\text{M}^{-1}\text{cm}^{-1}$ ).

120,000 = molar extinction coefficient (ε) at the longest-wavelength absorption maximum ( $\text{M}^{-1}\text{cm}^{-1}$ ).

0.51 = 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 paraformaldehyde 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.

## DATASHEET

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#### Mono anti-actin

#### Storage and Stability

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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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3 OF 3

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