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DATASHEET

Fluorescent Antibody F(ab) Atto488

Anti-Rabbit IgG F(ab)Atto488

Goat anti-Rabbit IgG F(ab)Atto488

For Use in Research Only.
Not for Use in Human or Veterinary Diagnostic or Therapeutic Processes.

Kit Content (Cat. #: 2312-250UG)

250µg Anti-Rabbit IgG F(ab)Atto488 (H+L)
1.0ml Glycerol buffer
Product datasheet

Product Documentation

Anti-Rabbit IgG F(ab)Atto488

Anti-Rabbit IgG F(ab)Atto488 is the antigen-specific fragment of the antibody obtained by papain cleavage. Affinity purification removed essentially all goat serum proteins, including immunoglobulins not specifically binding to rabbit IgG. F(ab) fragment was purified by ion exchange chromatography (IEX). Anti-Rabbit IgG F(ab) is conjugated to Atto488 NHS (Abs. max. 501 nm; Em. max. 523 nm) with a degree of labelling of 1-3 and subsequent purification by gel filtration. The antibody fragment is supplied in unit sizes of 250µg.

Reconstitution of Antibodies with Glycerol-PBS

Add 125µl Glycerol buffer to the lyophilized secondary antibody to reconstitute a 2mg/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, 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 – 1:500 are suited 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.

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

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.

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