

DATASHEET

Fluorescent Antibody F(ab')₂-Atto565

Anti-Mouse IgG F(ab')₂ Atto565

Goat anti-mouse IgG F(ab')₂ Atto565 (H+L)

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

Kit Content (Cat. #: 2407-0.5MG)

0.5mg Anti-Mouse IgG F(ab')₂ Atto565
1.0ml Glycerol buffer
Product datasheet

Product Documentation

Anti-Mouse IgG F(ab')₂ Atto565

Anti-mouse IgG F(ab')₂ Atto565 is the antigen-specific fragment of the antibody obtained by pepsin cleavage. Affinity purification removed essentially all goat serum proteins, including immunoglobulins not specifically binding to mouse IgG. F(ab')₂ fragment was purified by size exclusion chromatography (SEC). Anti-mouse IgG F(ab')₂ is conjugated to Atto565 NHS (Abs. max. 563 nm; Em.max. 592 nm) with a degree of labelling of 3-5 and subsequent purification by gel filtration. The antibody fragment is supplied in unit sizes of 500µg.

Reconstitution of Antibodies with Glycerol-PBS

Add 250µl 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 NaN₃ 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 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⁻¹cm⁻¹).

1 OF 2

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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.

$$\text{DOL} = \frac{A_{563} \cdot 203,000}{A_{280} - (A_{563} \cdot 0.16) \cdot 115,000}$$

A₅₆₃ = maximal absorbance at 563nm measured in a cuvette with a pathlength of 1 cm.

A₂₈₀ = 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⁻¹cm⁻¹).

120,000 = molar extinction coefficient (ε) at the longest-wavelength absorption maximum (M⁻¹cm⁻¹).

0.16 = 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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