

DATASHEET

Fluorescent Antibody Fab-Atto565

gam IgG (Fab) Atto565

Goat-anti mouse IgG (Fab) Atto565

For Use in Research Only.

Not for Use in Human or Veterinary
Diagnostic or Therapeutic Processes.

Kit Content (Cat. #: 2117-250UG)

250µg gam IgG (Fab) Atto565
Product documentation & Certificate of Analysis

Product Documentation

Goat anti-mouse IgG (Fab) Atto565

Goat anti-mouse IgG (Fab) Atto565 is the antigen-specific region fragment of the antibody. Affinity purification removed the Fc fragment including essentially all goat serum proteins, including immunoglobulins not specifically binding to mouse IgG. Goat anti-mouse IgG (Fab) is conjugated to Atto565 NHS (Abs.max. 563 nm; Em.max. 592 nm) and further purified by gel filtration. Goat anti-mouse IgG (Fab) Atto565 is supplied in unit sizes of 125µl (250µg).

Reconstitution of Antibodies with Glycerol-PBS (for freeze-dried shipped products only)

Add 0.125ml Glycerol-PBS to the freeze-dried 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 1:150 – 1:300 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⁻¹cm⁻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) \times molar concentration \times 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.

$$\text{DOL} = \frac{A_{563} \cdot 203,000}{A_{280} - (A_{563} \cdot 0.26) \cdot 115,000}$$

A_{563} = maximal absorbance at 563nm 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 ($\text{M}^{-1}\text{cm}^{-1}$).

0.16 = 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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