

## DATASHEET

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### Fluorescent Antibody F(ab)2-Atto594

#### Anti-Mouse IgG F(ab')<sub>2</sub> Atto594

Goat anti-mouse IgG F(ab')<sub>2</sub> Atto594

For Use in Research Only.

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

#### Kit Content (Cat. #: 2416-0.5MG)

0.5mg Anti-Mouse IgG F(ab')<sub>2</sub> Atto594 (H+L)  
1.0ml Glycerol buffer  
Product datasheet

#### Product Documentation

##### Anti-Mouse IgG F(ab')<sub>2</sub> Atto594

Anti-mouse IgG F(ab')<sub>2</sub> Atto594 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')<sub>2</sub> fragment was purified by size exclusion chromatography (SEC). Anti-mouse IgG F(ab')<sub>2</sub> is conjugated to Atto594 NHS (Abs. max. 601 nm; Em. max. 627 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 NaN<sub>3</sub> in 50% glycerol.

#### Working Dilution

Each individual user should determine the optimum working dilution empirically for the systems. Dilutions of 1:300 – 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 ( $\epsilon_{\text{max}} = 203,000 \text{ M}^{-1}\text{cm}^{-1}$ ).

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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 ( $\epsilon$ )  $\times$  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.

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

$A_{601}$  = 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 ( $\epsilon$ ) at the longest-wavelength absorption maximum ( $\text{M}^{-1}\text{cm}^{-1}$ ).

120,000 = molar extinction coefficient ( $\epsilon$ ) 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.

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