

## Anti-Hemagglutinin, Mouse Monoclonal 16B12, Alexa Fluor Conjugates (Anti-HA)

### Quick Facts

#### Storage upon receipt:

- 4°C or -20°C in aliquots
- Avoid freeze-thaw cycles
- Protect from light

**Working concentration:** 1–10 µg/mL

### Introduction

The anti-hemagglutinin mouse monoclonal antibody, clone 16B12 (IgG<sub>1,k</sub>), was raised against the twelve amino acid peptide CYPYDVDPDYASL. It recognizes the influenza hemagglutinin epitope YPYDVDPDYA, which has been used extensively as a general epitope tag in expression vectors.<sup>1</sup> The extreme specificity of this antibody allows for unambiguous identification of tagged proteins. Molecular Probes' Alexa Fluor<sup>®</sup> dye-labeled anti-HA antibodies<sup>2</sup> (Table 1) provide a convenient single-step immunolocalization methodology. This time-saving procedure will produce fluorescent images that have less background compared to the indirect staining methods commonly used. These antibodies are effective for detecting tagged proteins by immunoblotting or by fluorescence microscopy.

### Materials

#### Contents

The Alexa Fluor dye-labeled mouse anti-HA antibodies are supplied in unit sizes of 100 µg/mL as 1 mg/mL solutions in phosphate-buffered saline (PBS), pH 7.2, containing 5 mM azide and 0.1% bovine serum albumin (BSA).

### Reference

1. *Methods Enzymol* 194, pp. 508–519 (1991); 2. Unlabeled antibody provided by Covance Antibody Services, Inc.; 3. *Short Protocols in Molecular Biology*, 2<sup>nd</sup> Edition, F.M. Ausubel *et al.*, Eds., John Wiley and Sons (1992) pp. 14–24, 14–30.

Table 1. Alexa Fluor dye-labeled anti-HA monoclonal antibodies.

Catalog Number	Fluorophore	Abs *	Em *
A-21287	Alexa Fluor 488	495	519
A-21288	Alexa Fluor 594	590	617

\* Approximate absorption (Abs) and fluorescence emission (Em) maxima in nm for conjugates.

The degree of labeling for each conjugate is typically 2–8 fluorophore molecules per IgG molecule; the exact degree of labeling is indicated on the product label. At the time of preparation, the products are certified to be free of unconjugated dyes and are tested in a cytological experiment to ensure low nonspecific staining.

#### Storage

When these products are stored undiluted at 4°C and protected from light, they are stable for at least three months. For longer storage, divide the solution into single-use aliquots and freeze at -20°C. Frozen aliquots are stable for at least six months. PROTECT FROM LIGHT. AVOID REPEATED FREEZING AND THAWING.

### Application

It is a good practice to centrifuge the protein conjugate solution briefly in a microcentrifuge before use; only the supernatant should then be added to the experiment. This step will eliminate any protein aggregates that may have formed during storage, thereby reducing nonspecific background staining.

Because staining protocols vary with application, the appropriate dilution of antibody should be determined empirically. For fluorophore-labeled antibodies, a final concentration of 1–10 µg/mL should be satisfactory for most immunohistochemical applications.<sup>3</sup>

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**Product List** *Current prices may be obtained from our Web site or from our Customer Service Department.*

<b>Cat #</b>	<b>Product Name</b>	<b>Unit Size</b>
A-21287	anti-hemagglutinin, mouse IgG <sub>1</sub> , monoclonal 16B12, Alexa Fluor® 488 conjugate (anti-HA, Alexa Fluor® 488 conjugate) *1 mg/mL* .....	100 µL
A-21288	anti-hemagglutinin, mouse IgG <sub>1</sub> , monoclonal 16B12, Alexa Fluor® 594 conjugate (anti-HA, Alexa Fluor® 594 conjugate) *1 mg/mL* .....	100 µL

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