Anti–Fluorescein/Oregon Green® Antibodies and Conjugates

Quick Facts

Storage upon receipt:
- 4°C or −20°C in aliquots
- Avoid freeze-thaw cycles
- Protect fluorescent conjugates from light

Abs/Em of conjugates: See Table 1

Introduction

Anti–fluorescent dye antibodies recognize specific fluorophores and, in most cases, quench their fluorescence. Thus many anti-dye antibodies — including those that recognize fluorescein — can serve as cell-impermeant probes for determining whether fluorescent dye–conjugated ligands, proteins, bacteria or other biomolecules have been internalized by endocytic or pinocytic processes.1–4 In addition to their utility in quenching the fluorescence of extracellular fluorescein, the high affinity of Molecular Probes’ anti–fluorescein antibodies for both fluorescein and the structurally related Oregon Green® dye makes them ideal for immunochemical applications. Harmer and Samuel report the fluorescein–anti-fluorescein system provides a sensitive alternative to the biotin–streptavidin methods conventionally used for enzyme-linked immunosorbent assays (ELISAs).5 Fluorescein has also been found to be an excellent hapten for FISH, yielding sensitive results with extremely low background levels.6,7 Molecular Probes offers four unlabeled anti–fluorescein/Oregon Green antibodies (Table 1): a rabbit polyclonal IgG fraction, the Fab fragment of this rabbit polyclonal IgG, an affinity-purified goat polyclonal IgG fraction and a mouse monoclonal antibody. The purified Fab fragment provides researchers with a probe that more efficiently penetrates immunohistochemical preparations. Furthermore, the absence of the Fc region prevents binding to Fc receptor–bearing membranes. The mouse monoclonal antibody (clone 4-4-20)8 has been extensively used in studies focusing on mechanisms of antigen–antibody recognition.9–11 In addition to these unlabeled antibodies, we prepare biotin- and fluorophore-labeled conjugates of the rabbit and goat polyclonal anti–fluorescein/Oregon Green IgGs. The biotin-XX conjugate of anti–fluorescein can be used to convert fluorescence-based detection to an enzyme-amplified or electron microscopy technique, whereas the R-phycoerythrin, Alexa Fluor® 594 and Texas Red® dye–labeled antibodies can be used to transform fluorescence emission into red fluorescence. The Alexa Fluor 488 conjugates can be used to amplify the fluorescence signal while still allowing fluorescein-compatible optics.

Contents and Storage

Unlabeled Anti–Fluorescein/Oregon Green Polyclonal IgG Fractions

The rabbit and goat anti–fluorescein/Oregon Green IgGs (A-889, A-11095) are supplied in a unit size of 0.5 mL as a 1 mg/mL solution. The rabbit IgG is in 0.1 M potassium phosphate, pH 8, containing 5 mM sodium azide; the goat IgG is in phosphate-buffered saline (PBS), pH 7.2, containing 5 mM sodium azide. Molecular Probes has adopted a sensitive quenching assay to ensure that these antibodies are provided at a consistently high titer value. As supplied, 20 µL of the antibody solution is certified to produce ≥50% of the maximal fluorescence quenching of 1 mL of a 50 nM solution of fluorescein, assayed in 100 mM sodium phosphate, pH 8.0.Maximal quenching of fluorescein is ~90% of the fluorescence of the free dye. Due to steric hindrance, maximal fluorescence quenching of fluorescein covalently bound to protein may be significantly less.

When these products are stored undiluted at 4°C, 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. AVOID REPEATED FREEZING AND THAWING.

Unlabeled Anti–Fluorescein/Oregon Green Rabbit Polyclonal IgG Fab Fragment

The anti–fluorescein/Oregon Green Fab fragment (A-6413) is supplied in a unit size of 0.5 mL as a 0.5 mg/mL solution in 0.1 M
potassium phosphate, pH 8, containing 5 mM sodium azide. Molecular Probes uses a sensitive quenching assay (described above) to ensure that this antibody is provided at a consistently high titer value.

When this product is stored undiluted at 4°C, it is 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. AVOID REPEATED FREEZING AND THAWING.

**Unlabeled Anti–Fluorescein/Oregon Green Mouse Monoclonal Antibody**

The high-affinity mouse-monoclonal anti–fluorescein (clone 4-4-20, A-6421), which is purified from a hybridoma cell line made by chemically fusing murine myeloma cell line Sp2/0-Ag14 with splenocytes from BALB/cV mice, is supplied in a unit size of 0.5 mg as antibody lyophilized from a solution containing 0.1 M sodium phosphate, pH 8.0, 1.5% bovine serum albumin and 0.01% thimerosal. A stock solution can be made by dissolving the powder in 0.5 mL of 0.1 M potassium phosphate, pH 8, or 0.1 M sodium phosphate, 0.1 M NaCl, pH 7.5, as indicated on the label, and 5 mM sodium azide. The absorbance and fluorescence emission maxima of these conjugates are listed in Table 1.

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

**Fluorophore- and Biotin-Labeled Anti–Fluorescein/Oregon Green Polyclonal IgG Fractions**

The Alexa Fluor 488, Alexa Fluor 594 and biotin-XX anti–fluorescein/Oregon Green conjugates are supplied in unit sizes of

<table>
<thead>
<tr>
<th>Cat #</th>
<th>Product Name</th>
<th>Unit Size</th>
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</thead>
<tbody>
<tr>
<td>A-11096</td>
<td>anti-fluorescein/Oregon Green® goat IgG fraction, Alexa Fluor® 488 conjugate <em>1 mg/mL</em></td>
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<tr>
<td>A-11095</td>
<td>anti-fluorescein/Oregon Green® goat IgG fraction <em>1 mg/mL</em></td>
<td>0.5 mL</td>
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<td>A-6421</td>
<td>anti-fluorescein/Oregon Green®, mouse monoclonal 4-4-20</td>
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<td>A-6413</td>
<td>anti-fluorescein/Oregon Green®, rabbit IgG Fab fragment <em>0.5 mg/mL</em></td>
<td>0.5 mL</td>
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<tr>
<td>A-889</td>
<td>anti-fluorescein/Oregon Green®, rabbit IgG fraction <em>1 mg/mL</em></td>
<td>0.5 mL</td>
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<tr>
<td>A-11090</td>
<td>anti-fluorescein/Oregon Green®, rabbit IgG fraction, Alexa Fluor® 488 conjugate <em>1 mg/mL</em></td>
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<tr>
<td>A-11091</td>
<td>anti-fluorescein/Oregon Green®, rabbit IgG fraction, Alexa Fluor® 594 conjugate <em>1 mg/mL</em></td>
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<td>A-982</td>
<td>anti-fluorescein/Oregon Green®, rabbit IgG fraction, biotin-XX conjugate <em>1 mg/mL</em></td>
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<td>A-21250</td>
<td>anti-fluorescein/Oregon Green®, rabbit IgG fraction, R-phycoerythrin conjugate <em>2 mg/mL</em></td>
<td>250 µL</td>
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<td>A-981</td>
<td>anti-fluorescein/Oregon Green®, rabbit IgG fraction, Texas Red® conjugate <em>1 mg/mL</em></td>
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</tbody>
</table>

**References**


**Application**

Our anti-dye antibodies can be used in many different applications.12 Because staining protocols vary with application, the appropriate dilution of antibody should be determined empirically. Once that dilution is established, Molecular Probes’ quality control procedures ensure consistent performance from lot to lot.

It is a good practice to centrifuge the labeled antibody solutions 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.
Molecular Probes' products are high-quality reagents and materials intended for research purposes only. These products must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Please read the Material Safety Data Sheet provided for each product; other regulatory considerations may apply.

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