*Revised: 23–February–2001*

# *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 fluorescein emission into red fluorescence. The Alexa Fluor 488 conjugates can be used to amplify the fluorescence signal while still allowing fluorescein-compatible optics.

***Table 1.*** *Anti–fluorescein/Oregon Green antibodies and conjugates.*

| Cat # | Antibody | Label | Abs* | Em* |
|---|---|---|---|---|
| A-889 | Rabbit polyclonal IgG | None | NA | NA |
| A-6413 | Rabbit polyclonal IgG, Fab fragment | None | NA | NA |
| A-11095 | Goat polyclonal IgG | None | NA | NA |
| A-6421 | Mouse monoclonal (clone 4-4-20) | None | NA | NA |
| A-982 | Rabbit polyclonal IgG | Biotin | NA | NA |
| A-11090 | Rabbit polyclonal IgG | Alexa Fluor 488 | 495 | 519 |
| A-21250 | Rabbit polyclonal IgG | R-phycoerythrin | 565 | 578 |
| A-11091 | Rabbit polyclonal IgG | Alexa Fluor 594 | 590 | 617 |
| A-981 | Rabbit polyclonal IgG | Texas Red | 595 | 615 |
| A-11096 | Goat polyclonal IgG | Alexa Fluor 488 | 495 | 519 |

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

## *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,[8] 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 deionized water to yield an antibody concentration of 1 mg/mL.

When stored desiccated at 4°C or -20°C, the lyophilized product is stable for at least six months. Stock solutions are stable for at least three months when stored at 4°C. For longer storage of solutions, divide into aliquots and freeze at -20°C. Frozen aliquots are stable for at least six months. AVOID REPEATED FREEZING AND THAWING OF SOLUTIONS.

### *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 0.5 mL as 1 mg/mL solutions in 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 R-phycoerythrin conjugate is supplied in a 250 µL unit size as a 2 mg/ml solution in 0.1 M sodium phosphate, 0.1 M, NaCl, pH 7.5, 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.

## *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.

## *References*


**1.** Biochemistry 30, 2888 (1991); **2.** Biochim Biophys Acta 817, 238 (1985); **3.** Biochim Biophys Acta 778, 612 (1984); **4.** J Biol Chem 259, 5661 (1984); **5.** J Immunol Methods 122, 115 (1989); **6.** J Histochem Cytochem 38, 467 (1990); **7.** E.W. Voss Jr., *Fluorescein Hapten: An Immunological Probe*, CRC Press (1984); **8.** Mol Immunol 18, 889 (1981); **9.** Biochim Biophys Acta 1119, 27 (1992); **10.** Biochemistry 32, 10423 (1993); **11.** Biochemistry 32, 10929 (1993); **12.** Harlow, E. and Lane, D., *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press (1988).


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

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

## *Contact Information*

Further information on Molecular Probes' products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Leiden, the Netherlands. All others should contact our Technical Assistance Department in Eugene, Oregon.

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