

Anti–Lucifer Yellow and Anti–Alexa Fluor[®] 405/Cascade Blue[®] Antibodies

- A-5750** anti–lucifer yellow, rabbit IgG fraction
A-5751 anti–lucifer yellow, rabbit IgG fraction, biotin-XX conjugate
A-5760 anti–Alexa Fluor[®] 405/Cascade Blue[®], rabbit IgG fraction

Quick Facts

Storage upon receipt:

- 2–6°C or ≤–20°C in aliquots
- Avoid freeze-thaw cycles

Introduction

Molecular Probes offers a variety of anti–fluorescent dye antibodies that recognize specific fluorophores and, in most cases, quench their fluorescence. These anti-dye antibodies — including those that recognize the lucifer yellow, Alexa Fluor 405 and Cascade Blue fluorophores — 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, biotinylated conjugates can be used to convert fluorescence-based detection to an enzyme-amplified or electron microscopy technique.

Molecular Probes' anti–lucifer yellow (A-5750, A-5751) and anti–Alexa Fluor 405/Cascade Blue (A-5760) antibodies were specially developed to aid the neuroscientist. Lucifer yellow CH and the fixable Cascade Blue hydrazide are commonly used to characterize the morphology of neurons and to identify patterns of junctional communication.⁵ The fluorescence of a dye-filled neuron's finer processes, however, may be faint and fade rapidly or may be obscured by more intensely stained portions of the neuron. Our anti-dye antibodies have been used to overcome these limitations.^{6–9}

Materials

Anti–Lucifer Yellow (A-5750) and Biotin Anti–Lucifer Yellow (A-5751)

The anti–lucifer yellow antibody (A-5750) and its biotin-XX conjugate (A-5751) are supplied in unit sizes of 0.5 mL as 3 mg/mL solutions in phosphate-buffered saline (PBS), pH 7.2, containing 5 mM sodium azide. Molecular Probes uses a sensi-

tive quenching assay to ensure that these antibodies are provided at a consistently high titer value. As supplied, 20 µL of the antibody solutions are certified to produce ≥50% of the maximal fluorescence quenching of 1 mL of a 50 nM solution of lucifer yellow dye, assayed in 100 mM sodium phosphate, pH 8.0. Maximal quenching for lucifer yellow is ~85% of the fluorescence of the free dye. Due to steric hindrance, maximal fluorescence quenching of the lucifer yellow fluorophore covalently bound to protein may be significantly less.

Anti–Alexa Fluor 405/Cascade Blue (A-5760)

This antibody is supplied in a unit size of 0.5 mL as a 3 mg/mL solution in PBS, pH 7.2, containing 5 mM sodium azide. Molecular Probes uses a sensitive quenching assay to ensure that this antibody is provided at a consistently high titer value. As supplied, 20 µL of the antibody solutions are certified to produce ≥50% of the maximal fluorescence quenching of 1 mL of a 50 nM solution of Cascade Blue dye, assayed in 100 mM sodium phosphate, pH 8.0. Maximal quenching for Cascade Blue is ~80% of the fluorescence of the free dye. Due to steric hindrance, maximal fluorescence quenching of the Cascade Blue fluorophore covalently bound to protein may be significantly less. Cascade Blue and Alexa Fluor 405 are structurally similar; the antibody recognizes both fluorophores equally well.

Storage

When these products are stored undiluted at 2–6°C, 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.**

Application

Our anti-dye antibodies can be used in many different applications.¹⁰ Because staining protocols vary with application, the appropriate dilution of antibody should be determined empirically.

It is a good practice to centrifuge protein conjugate 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. Nature 292, 17 (1981); 6. J Neurosci 10, 1892 (1990); 7. J Neurosci Methods 36, 185 (1991); 8. Dev Biol 94, 391 (1982); 9. J Comp Neurol 296, 598 (1990); 10. 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-5760 | anti-Alexa Fluor® 405/Cascade Blue®, rabbit IgG fraction *3 mg/mL* | 0.5 mL |
| A-5750 | anti-lucifer yellow, rabbit IgG fraction *3 mg/mL* | 0.5 mL |
| A-5751 | anti-lucifer yellow, rabbit IgG fraction, biotin-XX conjugate *3 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.

Please visit our Web site — www.probes.com — for the most up-to-date information

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