Revised: 24–June–2004

Alexa Fluor® Hydrazides

Quick Facts

Storage upon receipt:

A10440, A10441, A10442:

- ≤–20°C
- Protect from light

A10436, A10437, A10438, A10439, A20501MP, A20502, A30634

- Room temperature
- Desiccate
- Protect from light

Table 1. *Molecular Probes' Alexa Fluor hydrazides.*

Hydrazide	Solid	10 mM Solution for Microinjection	Abs*	Em*
Alexa Fluor 350	A10439		345	445
Alexa Fluor 488	A10436	A10440	493	517
Alexa Fluor 555	A20501MP		554	567
Alexa Fluor 568	A10437	A10441	576	599
Alexa Fluor 594	A10438	A10442	588	613
Alexa Fluor 633	A30634		624	643
Alexa Fluor 647	A20502		649	666

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

Introduction

The Alexa Fluor® hydrazides offer a significant improvement over many conventional fluorophores for intracellular labeling experiments. The Alexa Fluor dyes are much brighter and more resistant to photobleaching, especially compared to fluorescein or lucifer yellow. The excitation spectra of the Alexa Fluor dyes closely matches the output of commonly used light sources (mercury-arc lamps and laser), they are readily visualized using common filter sets, they can be used with a variety of electrolytes and they can be iontophoretically injected using hyperpolarizing current. These properties make them the dye of choice for most intracellular applications.

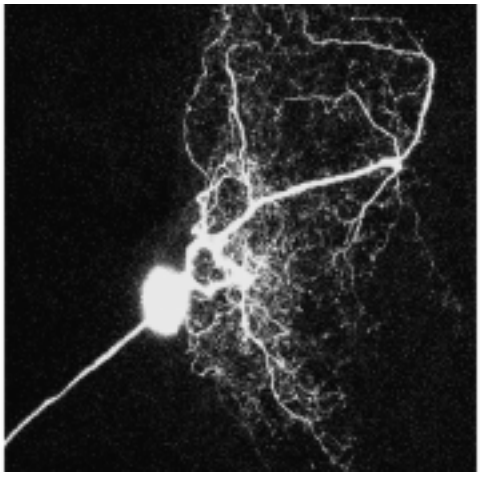

Figure 1. *The APR motor neuron of a larval moth,* Manduca sexta, *was labeled by the intracellular injection of Alexa Fluor 488 hydrazide (A10436). This pseudocolored image was created by combining 21 optical sections obtained with a scanning confocal microscope equipped with a bandpass filter appropriate for fluorescein. Photo contributed by Jack Gray, Institute of Neuroscience, University of Oregon, and Walter K. Metcalfe, Molecular Probes, Inc.*

Materials

Contents

Alexa Fluor hydrazides (Table 1) are now offered in solution, ready to inject into cells. Alexa Fluor 488 hydrazide (A10440), Alexa Fluor 568 hydrazide (A10441) and Alexa Fluor 594 hydrazide (A10442) are prepared as 10 mM solutions in 200 mM KCl and filter sterilized. Electrodes using these prepared solutions have conductances that are very nearly like those in 200 mM KCl alone, and are suitable for intracellular physiology, as well as for cell labeling.

Alexa Fluor hydrazides are also offered in solid form (Table 1) in a 1 mg unit size. Solutions may be prepared as needed if an experiment requires the use of electrolytes other than KCl (e.g., lithium chloride or potassium acetate). However, Alexa Fluor dyes may not be completely soluble in solutions of high ionic strength. For example, Alexa Fluor hydrazide 488 precipitates in 1 M potassium acetate at room temperature, but dissolves well in 200 mM potassium acetate.

Storage and Handling

The Alexa Fluor hydrazides in solutions should be stored at ≤–20°C, protected from light. The Alexa Fluor hydrazides as solids can be stored at room temperature, desiccated and protected from light. When stored properly, Alexa Fluor hydrazides, in either form, should remain stable for at least 6 months.

Applications

Cell Labeling

Intracellular labeling with 10 mM Alexa Fluor hydrazide solutions in 200 mM KCl is accomplished by passing hyperpolarizing current through the electrode. For example, to label the huge accessory planta retractor (APR) motoneurons in larvae of *Manduca sexta*, 2-3 nA DC hyperpolarizing current for 15 minutes worked well for an electrode with 50 megohm resistance (Figure 1). Smaller cells will require much less time to fill. The amount of time required for dye diffusion to label fine processes is similar to the time required for other commonly used dyes and depends on the distance and diameter of the processes.

Fixation

These dyes may be fixed using standard aldehyde fixation (e.g. 4% paraformaldehyde in 100 mM phosphate buffer, pH 7). However, we have observed that the fluorescence of Alexa Fluor 488 hydrazide is largely quenched after overnight fixation. Very bright labeling with excellent histological preservation was obtained with only 30 minutes of fixation at room temperature in the APR motoneurons mentioned above. Following fixation, the preparation may be dehydrated in ethanol and cleared in xylene. For the best long-term observation and storage, the fixed specimen may be mounted in an antifade medium such as ProLong® reagent (P7481).

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

Cat #	Product Name	Unit Size
A10439	Alexa Fluor® 350 hydrazide, sodium salt	5 mg
A10436	Alexa Fluor® 488 hydrazide, sodium salt	1 mg
A10440	Alexa Fluor® 488 hydrazide, sodium salt *for microinjection* *10 mM in 200 mM KCl*	125 µL
A20501MP	Alexa Fluor® 555 hydrazide, tris(triethylammonium salt)	1 mg
A10437	Alexa Fluor® 568 hydrazide, sodium salt	1 mg
A10441	Alexa Fluor® 568 hydrazide, sodium salt *for microinjection* *10 mM in 200 mM KCl*	125 µL
A10438	Alexa Fluor® 594 hydrazide, sodium salt	1 mg
A10442	Alexa Fluor® 594 hydrazide, sodium salt *for microinjection* *10 mM in 200 mM KCl*	125 µL
A30634	Alexa Fluor® 633 hydrazide, bis(triethylammonium salt)	1 mg
A20502	Alexa Fluor® 647 hydrazide, tris(triethylammonium salt)	1 mg

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

Molecular Probes, Inc.
29851 Willow Creek Road, Eugene, OR 97402
Phone: (541) 465-8300 ● Fax: (541) 335-0504

Customer Service: 6:00 am to 4:30 pm (Pacific Time)
Phone: (541) 335-0338 ● Fax: (541) 335-0305 ● order@probes.com

Toll-Free Ordering for USA and Canada:
Order Phone: (800) 438-2209 ● Order Fax: (800) 438-0228

Technical Assistance: 8:00 am to 4:00 pm (Pacific Time)
Phone: (541) 335-0353 ● Toll-Free: (800) 438-2209
Fax: (541) 335-0238 ● tech@probes.com

Molecular Probes Europe BV
Poortgebouw, Rijnsburgerweg 10
2333 AA Leiden, The Netherlands
Phone: +31-71-5233378 ● Fax: +31-71-5233419

Customer Service: 9:00 to 16:30 (Central European Time)
Phone: +31-71-5236850 ● Fax: +31-71-5233419
eurorder@probes.nl

Technical Assistance: 9:00 to 16:30 (Central European Time)
Phone: +31-71-5233431 ● Fax: +31-71-5241883
eurotech@probes.nl