SelectFX® Alexa Fluor® 488 Peroxisome Labeling Kit

Catalog no. S34201

Table 1. Contents and storage information.

Material	Amount	Concentration	Storage	Stability
SelectFX® Alexa Fluor® 488 Peroxisome Labeling Kit 2–6˚C Components				
Anti-peroxisomal membrane protein 70 (PMP 70) rabbit IgG fraction* (Component A)	50 µL	250 µg/mL solution in PBS, 2 mM sodium azide	• 2–6˚C • Protect from light • DO NOT FREEZE	When stored as directed, the components are stable for up to 3 months.
Alexa Fluor® 488 goat anti–rabbit IgG (H+L), highly cross-adsorbed* (Component B)	50 µL	2 mg/mL solution in 0.1 M sodium phosphate, 0.1 NaCl, pH 7.5, 5 mM sodium azide		
Phosphate-buffered saline (PBS)* (Component C)	100 mL	10X		
Blocking reagent (Component D)*	50 mL	10X, 100% heat-inactivated normal goat serum (NGS)		
SelectFX® Kits 2–25˚C Components				
Fixative solution (Component A)	2 glass ampules (10 mL each)	4X, methanol-free 16% formaldehyde solution	• 2–25˚C • DO NOT FREEZE	When stored as directed, the components are stable for at least 6 months
Permeabilization solution (Component B)	1.25 mL	100X, 20% Triton® X-100 solution		
* For long-term storage, any of these components may be divided into aliquots and stored at ≤–20˚C. Avoid repeated freezing and thawing of components stored at ≤–20˚C.				
Approximate fluorescence excitation/emission maxima: Alexa Fluor® 488 dye conjugate: ~495/519 nm. The labeling can be observed using standard fluorescein filter sets.				

Introduction

Peroxisomes are single membrane-bound vesicles that are found in most eukaryotic cells. Their chief function is to enzymatically oxidize fatty acids and to subsequently catalyze the breakdown of H_2O_2, a by-product of fatty acid degradation. Recently, interest in peroxisomes has increased especially in studies related to peroxisomal origin and maintenance.[1] Morphological abnormalities in peroxisomes related to disease states and diet have also been on the forefront of current research.[2,3]

The SelectFX® Alexa Fluor® 488 Peroxisome Labeling Kit provides all the reagents needed to label the peroxisomes in fixed cells, and includes cell fixative and permeabilization reagents. For detection, the kit uses an antibody directed against peroxisomal membrane protein 70 (PMP 70), which is a high-abundance integral-membrane component of peroxisomes.[4] PMP 70 is significantly induced by administration of hypolipidemic agents in parallel with peroxisome proliferation and the induction of peroxisomal fatty acid beta-oxidation enzymes.[4]

Before You Begin

Cautions

Fixative Solution (Component A in SelectFX® Kits 2–25˚C Components): Contains 18% formaldehyde, and is harmful by inhalation, in contact with skin, and if swallowed. Irritating to eyes, respiratory system, and skin. Limited evidence of a carcinogenic effect. May cause sensitization by skin contact. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible). Use only in well ventilated areas. Wear suitable protective clothing, gloves, and eye/face protection when handling this reagent.

Permeabilization Solution (Component B in SelectFX® Kits 2–25˚C Components): Contains Triton® X-100, and is an irritant. Risk of serious damage to eyes. Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection when handling this reagent. Avoid release to the environment. Refer to special instructions/safety data sheets.

Preparing Working Solutions

You can prepare the working solutions by mixing the entire contents of the supplied stock solutions at once, or on a per assay basis. Both methods are described below.

1.1. Prepare 1X PBS. Mix 100mL of 10X PBS (Component C) and 900 mL of deionized water (dH_2O) to make a 1 liter of 1X PBS solution. For single assay preparation, add 1.0 mL of 10X PBS to 9.0 mL of dH_2O to make 10 mL of 1X PBS. Note that a portion of this solution will be used to prepare other working solutions and the remainder will be used as a wash buffer. If you are using the kit for the first time, prepare an additional 30 mL of 1X PBS for use in making up the 1X fixative solution (see step 1.2). Store unused PBS at 2–6°C.

1.2. Prepare the 1X fixative solution. We recommend that you use the entire contents of the ampule to make the working solution (we do not recommend preparing small amounts of fixative solution for each assay). In a separate container, add the contents of one of the two supplied 10 mL ampules of 4X fixative solution (Component A in SelectFX® Kits 2–25˚C Components) to 30 mL of 1X PBS (prepared in step 1.1) to make a 4% fixative solution. Do not open the second ampule until you need to prepare more 1X fixative solution. Store unused 1X fixative solution at room temperature.

Note: The vial is designed to break at the narrow, scored neck. Exercise extreme care when

opening the glass ampule of 4X fixative solution. First, hold the ampule vertically and tap it gently to ensure all of the fixative solution is in the body of the ampule. Then, using appropriate safety equipment to protect your hands and face, hold the ampule vertically and snap off the top.

1.3 Prepare the 1X permeabilization solution. In a separate glass container, mix 1 mL of the 100X permeabilization solution (Component B in SelectFX® Kits 2–25˚C Components) and 99 mL of 1X PBS (prepared in step 1.1) to make a 1X permeabilization solution of 0.2% Triton® X-100. For single assay preparation, add 10 µL of the 100X permeabilization solution to 990 µL of 1X PBS. Store unused permeabilization solution at room temperature.

1.4 Prepare the 1X blocking reagent. In a separate container, mix 50 mL of the 10X blocking reagent (Component D in SelectFX® Alexa Fluor® Peroxisome Labeling Kit 2–6˚C Components) and 450 mL of 1X PBS (prepared in step 1.1) to make a 1X blocking reagent consisting of 10% NGS. For single assay preparation, add 300 µL of 10X blocking reagent to 2.7 mL of 1X PBS. Store unused blocking reagent at 2–6°C.

Experimental Protocol

This protocol was developed using bovine pulmonary artery endothelial (BPAE) cells on coverslips but is broadly adaptable to other cell lines. Adjust the experimental parameters such as the amount of antibody used for staining and the incubation time to achieve optimal staining. You may also adapt this protocol for multicolor staining in conjunction with other probes for cellular targets.

You may perform the protocol below, *Peroxisome Staining*, using adherent cells grown on a coverslip. If you are using nonadherent cells, deposit the washed cells onto a slide prior to staining.

Centrifuge concentrated primary and secondary antibody solutions at ~10,000 × *g* for ~2 minutes at 4°C to sediment invisible aggregates before taking an aliquot for dilution (steps 2.7 and 2.9).

Peroxisome Staining

2.1 Wash the cells. Warm 1X PBS (prepared in step 1.1) to 37°C. Wash the cells once using 1.0 mL of warmed 1X PBS.

2.2 Fix the cells. Apply 0.8 mL of the 1X fixative solution (prepared in step 1.2) to the sample. Incubate for 15 minutes at 37°C.

2.3 Wash the cells. Wash the cells with 1.0 mL of room temperature 1X PBS. Repeat the wash once.

2.4 Permeabilize the cells. Apply 1.0 mL of 1X permeabilization solution (prepared in step 1.3) to the sample. Incubate the sample at room temperature for 5 minutes.

2.5 Wash the cells. Wash the cells with 1.0 mL of room temperature 1X PBS. Repeat the wash once.

2.6 Apply blocking solution. Apply 1.0 mL of 1X blocking reagent (prepared in step 1.4) to the sample. Incubate the sample for 30–60 minutes at room temperature.

2.7 Apply the diluted primary antibody solution to the sample. Prepare a 1,000-fold dilution of the anti–PMP 70 antibody by centrifuging the tube containing the anti–PMP 70 antibody and adding 1.0 µL of the antibody solution to 1.0 mL of 1X blocking reagent (prepared in step 1.4). Mix well, add the diluted antibody solution to the sample, and incubate at room temperature for 1–2 hours.

2.8 Wash the cells with 1.0 mL of 1X blocking solution. Repeat the wash 3–4 times.

2.9 Apply the diluted secondary antibody solution to the sample. Prepare a 1,000-fold dilution of the Alexa Fluor® 488–labeled secondary antibody by centrifuging the tube containing the secondary antibody, and adding 1.0 µL of the antibody solution to 1.0 mL of 1X PBS. Mix well, add the diluted secondary antibody staining solution to the sample, and incubate at room temperature for 30 minutes protected from light.

2.10 Wash the cells with 1.0 mL of 1X PBS. Repeat the wash 3–4 times.

2.11 If desired, counterstain the cells with DAPI or another nucleic acid stain.

2.12 Mount the cells. For best results, use an antifade reagent such as ProLong® Gold antifade reagent. View the sample with a fluorescence microscope equipped with filters appropriate for fluorescein.

References

1. Mol Biol Cell 14, 2900 (2003); **2.** Am J Hum Genet 73, 233 (2003); **3.** Mar Environ Res 54, 297 (2002); **4.** Cell Biochem Biophys 32, 131 (2000).

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

Cat. no.	Product Name	Unit Size
S34201	SelectFX® Alexa Fluor® 488 Peroxisome Labeling Kit *for fixed cells*	1 kit
Related products		
A11034	Alexa Fluor® 488 goat anti-rabbit IgG (H+L) *highly cross-adsorbed* *2 mg/mL*	0.5 mL
P36930	ProLong® Gold antifade reagent	10 mL
P36931	ProLong® Gold antifade reagent with DAPI	10 mL
P36934	ProLong® Gold antifade reagent *special packaging*	5 x 2 mL
P36935	ProLong® Gold antifade reagent with DAPI *special packaging*	5 x 2 mL

Contact 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
probesorder@invitrogen.com

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

Technical Service:
8:00 am to 4:00 pm (Pacific Time)
Phone: (541) 335-0353
Toll-Free (800) 438-2209
Fax: (541) 335-0238
probestech@invitrogen.com

Invitrogen European Headquarters
Invitrogen, Ltd.
3 Fountain Drive
Inchinnan Business Park
Paisley PA4 9RF, UK
Phone: +44 (0) 141 814 6100
Fax: +44 (0) 141 814 6260
Email: euroinfo@invitrogen.com
Technical Services: eurotech@invitrogen.com

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 Paisley, United Kingdom. All others should contact our Technical Assistance Department in Eugene, Oregon.

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.

Limited Use Label License No. 223: Labeling and Detection Technology
The manufacture, use, sale or import of this product may be subject to one or more patents or pending applications owned or licensed by Invitrogen Corporation. The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity) in a manner consistent with the accompanying product literature. The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes. The buyer may transfer information or materials made through the use of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing (a) to not transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for Commercial Purposes. Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. For products that are subject to multiple limited use label licenses, the most restrictive terms apply. Invitrogen Corporation will not assert a claim against the buyer of infringement of patents that are owned or controlled by Invitrogen Corporation and/or Molecular Probes, Inc. which cover this product based upon the manufacture, use or sale of a therapeutic, clinical diagnostic, vaccine or prophylactic product developed in research by the buyer in which this product or its components was employed, provided that neither this product nor any of its components was used in the manufacture of such product. If the purchaser is not willing to accept the limitations of this limited use statement, Invitrogen is willing to accept return of the product with a full refund. For information on purchasing a license to this product for purposes other than research, contact Molecular Probes, Inc., Business Development, 29851 Willow Creek Road, Eugene, OR 97402, Tel: (541) 465-8300. Fax: (541) 335-0354.

Several Molecular Probes products and product applications are covered by U.S. and foreign patents and patents pending. All names containing the designation ® are registered with the U.S. Patent and Trademark Office.