SelectFX® Alexa Fluor® 488 Peroxisome Labeling Kit

Catalog no. S34201

<table>
<thead>
<tr>
<th>Material</th>
<th>Amount</th>
<th>Concentration</th>
<th>Storage</th>
<th>Stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>SelectFX® Alexa Fluor® 488 Peroxisome Labeling Kit 2–6°C Components</td>
<td></td>
<td></td>
<td></td>
<td>When stored as directed, the components are stable for up to 3 months.</td>
</tr>
<tr>
<td>Anti-peroxisomal membrane protein 70 (PMP 70) rabbit IgG fraction* (Component A)</td>
<td>50 µL</td>
<td>250 µg/mL solution in PBS, 2 mM sodium azide</td>
<td>• 2–6°C • Protect from light • DO NOT FREEZE</td>
<td></td>
</tr>
<tr>
<td>Alexa Fluor® 488 goat anti–rabbit IgG (H+L), highly cross-adsorbed* (Component B)</td>
<td>50 µL</td>
<td>2 mg/mL solution in 0.1 M sodium phosphate, 0.1 NaCl, pH 7.5, 5 mM sodium azide</td>
<td></td>
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</tr>
<tr>
<td>Phosphate-buffered saline (PBS)* (Component C)</td>
<td>100 mL</td>
<td>10X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blocking reagent (Component D)*</td>
<td>50 mL</td>
<td>10X, 100% heat-inactivated normal goat serum (NGS)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SelectFX® Kits 2–25°C Components</td>
<td></td>
<td></td>
<td></td>
<td>When stored as directed, the components are stable for at least 6 months</td>
</tr>
<tr>
<td>Fixative solution (Component A)</td>
<td>2 glass ampules (10 mL each)</td>
<td>4X, methanol-free 16% formaldehyde solution</td>
<td>• 2–25°C • DO NOT FREEZE</td>
<td></td>
</tr>
<tr>
<td>Permeabilization solution (Component B)</td>
<td>1.25 mL</td>
<td>100X, 20% Triton® X-100 solution</td>
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</tbody>
</table>

* 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 \( \text{H}_2\text{O}_2 \), a by-product of fatty acid degradation. Recently, interest in peroxisomes has increased especially in studies related to peroxisomal origin and maintenance. Morphological abnormalities in peroxisomes related to disease states and diet have also been on the forefront of current research.

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. 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.

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\(_2\)O) 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\(_2\)O 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.

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**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.

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**References**


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

<table>
<thead>
<tr>
<th>Cat. no.</th>
<th>Product Name</th>
<th>Unit Size</th>
</tr>
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<tbody>
<tr>
<td>S34201</td>
<td>SelectFX® Alexa Fluor® 488 Peroxisome Labeling Kit <em>for fixed cells</em></td>
<td>1 kit</td>
</tr>
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**Related products**

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<th>Product Name</th>
<th>Unit Size</th>
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<tbody>
<tr>
<td>A11034</td>
<td>Alexa Fluor® 488 goat anti-rabbit IgG (H+L) <em>highly cross-adsorbed</em> <em>2 mg/mL</em></td>
<td>0.5 mL</td>
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<tr>
<td>P36930</td>
<td>ProLong® Gold antifade reagent</td>
<td>10 mL</td>
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<td>P36931</td>
<td>ProLong® Gold antifade reagent with DAPI</td>
<td>10 mL</td>
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<tr>
<td>P36934</td>
<td>ProLong® Gold antifade reagent <em>special packaging</em></td>
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</tr>
<tr>
<td>P36935</td>
<td>ProLong® Gold antifade reagent with DAPI <em>special packaging</em></td>
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