

SelectFX® Alexa Fluor® 488 Cytochrome c Apoptosis Detection Kit

Table 1. Contents and storage information.

Material	Amount	Concentration	Storage	Stability
S43159 SelectFX® Alexa Fluor® 488 Cytochrome c Apoptosis Detection Kit (S35115) 2–6°C Components				
Anti-cytochrome c, mouse IgG*	100 µL	500 µg/mL solution in PBS, 2 mM sodium azide	<ul style="list-style-type: none"> • 2–6°C • Protect from light • DO NOT FREEZE 	Components stable for up to 3 months.
Alexa Fluor® 488 goat anti-mouse IgG (H+L), highly cross-adsorbed*	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)*	100 mL	10X		
Blocking solution*	50 mL	10X, 100% heat-inactivated normal goat serum (NGS)		
S34252 SelectFX® Kits 2–25°C Components				
Fixative solution	2 glass ampules (10 mL each)	4X, methanol-free 16% formaldehyde solution	<ul style="list-style-type: none"> • 2–25°C • DO NOT FREEZE 	Components stable for ~6 months.
Permeabilization solution	1.25 mL	100X, 20% solution of Triton X-100		
S34160 SelectFX® Alexa Fluor® 488 Cytochrome c Apoptosis Detection Kit (S35115) Product Info Sheet				
* 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: ~495/519 nm for the Alexa Fluor® 488 dye conjugate. The labeling can be observed using standard fluorescein filter sets				

Introduction

Cytochrome *c* is a ~12.3 kDa protein found in the mitochondrial intermembrane compartment and is associated with the inner membrane. Cytochrome *c* transports electrons from Complex III (cytochrome *c* reductase) to Complex IV (cytochrome *c* oxidase) in the mitochondrial respiratory chain, crucial for synthesis of ATP via oxidative phosphorylation. Of the many pro-apoptotic proteins in the mitochondria, cytochrome *c* is the most prominent, and release of cytochrome *c* from the mitochondrial inter-membrane compartment into the cytosol occurs in relatively early stages of apoptosis. Cytochrome *c* is associated with the mitochondrial inner membrane via an electrostatic attachment to cardiolipin. Peroxidation of cardiolipin is thought to result in detachment of cytochrome *c* from the inner membrane. If detachment is accompanied by Bax-mediated outer membrane permeabilization, cytochrome *c* is extruded from mitochondrion.¹⁻³ Once in the cytosol, cytochrome *c* drives the formation of the high molecular weight caspase-activating complex, the apoptosome.⁴⁻⁷

The SelectFX® Alexa Fluor® 488 Cytochrome *c* Apoptosis Detection Kit provides all the reagents needed to determine the location of cytochrome *c* in fixed cells. The kit employs an anti-cytochrome *c* primary antibody and an Alexa Fluor® 488 dye-labeled secondary antibody; fluorescence is observed using standard fluorescein filters. The kit also includes cell fixative and permeabilization reagents and protocols for mammalian cell preparation and staining.

Before You Begin

Preparing Working Solutions

The working solutions can be prepared 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. To a one-liter container, mix 100 mL of 10X PBS and 900 mL of deionized water (dH₂O) to make a 1X PBS solution. For single assay preparation, add 1.0 mL of 10X PBS to 9.0 mL of dH₂O 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 the kit is being used 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. It is recommended that the entire contents of the ampule be used to make the working solution (preparing small amounts of fixative solution for each assay is not recommended). In a separate container, add the contents of one of the two supplied 10 mL ampules of 4X fixative solution to 30 mL of 1X PBS (prepared in step 1.1) to make a 4% fixative solution. The second ampule need not be opened until more 1X fixative solution needs to be made. 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 1.0 mL of the 100X permeabilization solution to 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 1X PBS. Store unused permeabilization solution at room temperature.

1.4 Prepare the 1X blocking solution. In a separate container, mix 50 mL of the 10X blocking solution and 450 mL of 1X PBS (prepared in step 1.1) to make a 1X blocking solution consisting of 10% NGS. For single assay preparation, add 300 µL of 10X blocking solution to 2.7 mL of 1X PBS. Store unused blocking solution 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. Experimental parameters such as the amount of antibody used for staining and the incubation should be adjusted to achieve optimal staining. This protocol can also be adapted for use in conjunction with other probes for cellular targets for multicolor staining.

The protocol below, *Cytochrome c Labeling*, can be performed using adherent cells grown on a coverslip. If nonadherent cells are used, deposit the washed cells onto a slide prior to staining.

Concentrated primary and secondary antibody solutions should be centrifuged at ~10,000 g for ~2 minutes at 4°C to sediment invisible aggregates before an aliquot is taken for the dilution (steps 2.7 and 2.9).

Cytochrome c Labeling

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.

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.

2.6 Apply blocking solution. Apply 1.0 mL of 1X blocking solution (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 500-fold dilution of the anti-cytochrome *c* antibody by centrifuging the tube containing the anti-cytochrome *c* antibody and adding 1.0 µL of the antibody solution to 1.0 mL of 1X blocking solution (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 other 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. Proc Natl Acad Sci U S A 99, 1259 (2002); 2. Exp Cell Res 256, 50 (2000); 3. Science 281, 1322 (1998); 4. Cell 91, 479 (1997); 5. Nature 407, 770 (2000); 6. J Cell Physiol 192, 131 (2002); 7. J Biol Chem 278, 8091 (2003).

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

Cat #	Product Name	Unit Size
A11029	Alexa Fluor® 488 goat anti-mouse 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
S35115	SelectFX® Alexa Fluor® 488 Cytochrome c Apoptosis Detection Kit *for fixed cells*	1 kit

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