SelectFX® Nuclear Labeling Kit *for fixed cells*

Table 1. Contents and Storage Information.

Material	Amount	Concentration	Storage	Stability
DAPI, blue-fluorescent counterstain (Component A)	100 µL	300X, 180 µM solution in water	• ≤–20°C * • Protect from light • Desiccate • Store vials in an upright position	When stored as directed, stock reagents are stable for at least 6 months.
SYTOX® Green, green-fluorescent counterstain (Component B)	100 µL	300X, 50 µM solution in DMSO		
7-Aminoactinomycin D (7-AAD), red-fluorescent counterstain (Component C)	600 µL	50X, 1.58 mM solution in DMSO		
TO-PRO®-3 iodide, far red–fluorescent counterstain (Component D)	100 µL	300X, 300 µM solution in DMSO		
* Allow the reagents to warm to room temperature before opening the vials. Before refreezing, seal the vials tightly.				
Number of labelings: The SelectFX™ Nuclear Labeling Kit contains sufficient reagents to prepare ~100 assays with each stain at 300 µL per assay.				
Spectral Data: DAPI 358/461 nm; SYTOX® Green dye 504/523 nm; 7-AAD 546/647 nm; TO-PRO®-3 dye 642/661 nm.				

Introduction

The SelectFX® Nuclear Labeling Kit (S33025) provides four spectrally distinct fluorescent dyes for staining nuclei in fixed-cell preparations: blue-fluorescent DAPI, green-fluorescent SYTOX® Green stain, red-fluorescent 7-aminoactinomycin D (7-AAD), and far red–fluorescent TO-PRO®-3 dye. These dyes are ideal for use as counterstains in multicolor applications; simply select the stain that contrasts spectrally with other fluorescent probes applied to the sample. When used according to the protocol provided, the dyes in the SelectFX® Nuclear Labeling Kit provide highly selective nuclear staining with little or no cytoplasmic labeling. The stained nuclei stand out in vivid contrast to other fluorescently labeled cell structures when observed by fluorescence microscopy. These dyes have excitation wavelengths that match the common laser lines for confocal microscopy and flow cytometry and can be used with standard filter sets on fluorescence microscopes and microplate readers.

The staining protocol provided is compatible with a wide range of cytological labeling techniques, including direct or indirect antibody-based detection methods, mRNA *in situ* hybridization, or staining with fluorescent reagents specific for cellular structures (e.g., Molecular Probes' MitoTracker® Red CMXRos and Alexa Fluor® conjugated phalloidins). The dyes can also be used to fluorescently stain cells for analysis in multicolor flow cytometry experiments.

All dyes are provided as stock solutions, convenient for diluting and staining. Each dye is also available separately.

Caution SYTOX® Green stain, 7-AAD, and TO-PRO®-3 dye bind nucleic acids and should be treated as potential mutagens; DAPI is a known mutagen. Handle these reagents with appropriate caution and use safety equipment and practices appropriate for the hazards posed by these chemicals. DMSO stock solutions should be handled with particular caution as DMSO is known to facilitate the entry of organic molecules into tissues. Dispose of these reagents in compliance with all pertaining local regulations.

Experimental Protocol for Fluorescence Microscopy

The protocol outlined below has been optimized for fixation in 4% formaldehyde solutions using bovine pulmonary artery epithelial cells, but is compatible with other cell types. Other fixation techniques may result in nonspecific staining or abnormal cellular morphology. RNase treatment is not necessary, but could improve nuclear signals over cytoplasmic RNA background with SYTOX® Green or TO-PRO®-3 dye under some conditions, particularly if a higher concentration of dye is needed. If the dyes are to be used as nuclear counterstains, labeling steps involving other probes should be carried out first. DAPI and TO-PRO®-3 dyes provide optimum performance when prepared in phosphate-buffered saline (PBS), whereas SYTOX® Green dye and 7-AAD are best prepared in distilled water. Other buffers can be used, but cytoplasmic and nonspecific background may increase. Please see the product information sheets for the individual products (available at probes.invitrogen.com) to determine the best combinations of probes and counterstains.

General Staining Protocol

1.1 Fix cells. Fix adherent or suspension cells using 4% formaldehyde in complete media for 15 minutes at 37˚C.

1.2 Wash cells. Wash cells for 5 minutes in PBS; repeat twice.

1.3 Permeabilize cells. Permeabilize cells for 10 minutes with 0.2% Triton X-100 in PBS.

1.4 Rinse cells. Rinse cells well with PBS.

1.5 (Optional) Label non-nuclear structures. If other stains will be used, proceed with those label and wash steps.

1.6 Apply counterstain. When ready to stain nuclei, follow the guidelines for the dye used:

DAPI: Dilute DAPI stock solution (Component A) 1:300 into PBS to make a 0.2 µg/mL (600 nM) solution. Apply a sufficient amount of the 600 nM solution to cover cells, then incubate for 2 minutes. Proceed to Step 1.7.

SYTOX® Green stain: Dilute SYTOX® Green dye stock solution (Component B) 1:300 into water to make a 0.2 µg/mL (167 nM) solution. Rinse cells in water, then apply a sufficient amount of the 167 nM solution to cover cells. Incubate for 15 minutes, then rinse again with water before proceeding to the final washes in Step 1.7.

7-AAD: Dilute 7-AAD stock solution (Component C) 1:50 into water to make a 40 µg/mL (32 µM) solution. Rinse cells in water, then apply a sufficient amount of the 32 µM solution to cover cells. Incubate for 45 minutes, then rinse again with distilled water before proceeding to the final washes in Step 1.7.

TO-PRO®-3 dye:: Dilute TO-PRO®-3 dye stock solution (Component D) 1:300 into PBS to make a 0.7 µg/mL (1 µM) solution. Apply a sufficient amount of the 1 µM solution to cover cells, then incubate for 15 minutes. Proceed to Step 1.7.

1.7 Wash cells. Wash cells for 5 minutes in PBS; repeat twice.

1.8 Prepare for viewing. Mount coverslip in an appropriate antifade medium such as ProLong® Gold antifade reagent (P36930, P36934) or *Slowfade®* Gold antifade reagent (S36936, S36937)

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

Cat #	Product Name	Unit Size
S33025	SelectFX® Nuclear Labeling Kit *DAPI,SYTOX® Green,7-AAD,TO-PRO®-3 iodide* *for fixed cells*	1 kit
D1306	4′,6-diamidino-2-phenylindole, dihydrochloride (DAPI)	10 mg
S7020	SYTOX® Green nucleic acid stain *5 mM solution in DMSO*	250 µL
A1310	7-aminoactinomycin D (7-AAD)	1 mg
T3605	TO-PRO®-3 iodide (642/661) *1 mM solution in DMSO*	1 mL
P36930	ProLong® Gold antifade reagent	10 mL
P36934	ProLong® Gold antifade reagent *special packaging*	5 X 2 mL
S36936	SlowFade® Gold antifade reagent	10 mL
S36937	SlowFade® Gold antifade reagent *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.