SYTO® RNASelect™ Green Fluorescent Cell Stain (S32703)

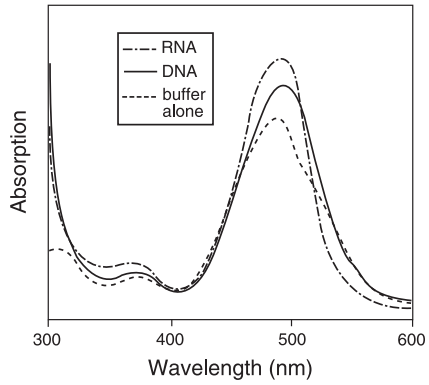
Quick Facts

Storage upon receipt:

- ≤–20ºC
- Protect from light
- Desiccate

Abs/Em: ~490/530 nm, bound to RNA

Concentration: 5 mM solution in DMSO



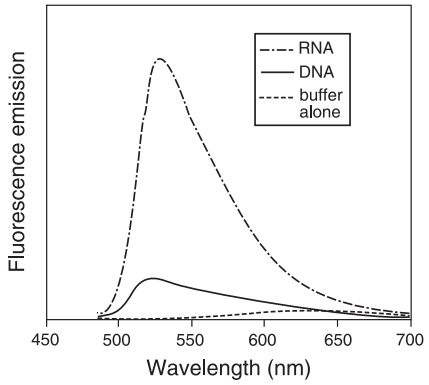


Figure 1. Relative absorption (A) and fluorescence emission (B) spectra of SYTO RNASelect green fluorescent cell stain in the presence of *E. coli* RNA or *E. coli* DNA, or in buffer alone.

Introduction

The SYTO® RNASelect™ green fluorescent cell stain is a cell-permeant nucleic acid stain that is selective for RNA. Although virtually nonfluorescent in the absence of nucleic acids, the SYTO RNASelect stain exhibits bright green fluorescence when bound to RNA (absorption/emission maxima ~490/530 nm) and only a weak fluorescent signal when bound to DNA (Figure 1). Eukaryotic cells stained with this green-fluorescent, RNA-selective dye can be observed by fluorescence microscopy using standard fluorescein filter sets.

Eukaryotic cells stained with the SYTO RNASelect dye show a staining pattern consistent with that of an RNA-selective probe.[1] Maximal fluorescence is observed in the nucleoli, with faint fluorescence throughout the nucleus. Weak fluorescence is also seen throughout the cytoplasm, predominantly associated with mitochondria. The RNA localization of the SYTO RNASelect stain is further supported by RNase and DNase treatments: 1) upon treatment with RNase, the nucleolar fluorescence signal is completely lost, and the nuclear and cytoplasmic intensities are significantly reduced as compared to control cells; 2) upon treatment with DNase, there is no significant loss of fluorescence; 3) upon treatment with both RNase and DNase, the staining pattern is the same as that observed with RNase treatment alone (Molecular Probes, unpublished results).

Because the SYTO RNASelect green fluorescent cell stain is cell permeant, it is suitable for staining live cells. After the cells have been stained, they may be fixed in methanol with minimal loss of the staining pattern. Fixation with formaldehyde, however, alters the staining pattern and is not recommended. If desired, cells can be fixed in methanol before staining for RNA with the SYTO RNASelect stain.

Materials

Contents

SYTO RNASelect green fluorescent cell stain is supplied in a unit size of 100 µL as a 5 mM solution in dimethylsulfoxide (DMSO).

Storage and Handling

Upon receipt, store the product at ≤–20°C, desiccated and protected from light. Note: Before opening, allow the product to warm to room temperature, and then briefly centrifuge it in a microcentrifuge to deposit the DMSO solution in the bottom of the vial. Before refreezing, seal the vial tightly. When stored properly, the DMSO solution should be stable for at least 1 year.

Caution: No data are available addressing the mutagenicity or toxicity of this reagent. Because the reagent binds to nucleic acids, it should be treated as a potential mutagen and used with appropriate care. The DMSO stock solution should be handled with particular caution, as DMSO is known to facilitate the entry of organic molecules into tissues. Please dispose of the stain in compliance with all pertaining local regulations.

Spectral Characteristics

Filter sets suitable for fluorescein (FITC) are recommended for imaging SYTO RNASelect green fluorescent cell stain. The peak excitation and emission of the dye is approximately 490/530 nm, respectively, when bound to RNA (Figure 1). NOTE: Some excitation and emission of this dye can occur when the dye is observed with standard tetramethylrhodamine (TRITC) filter sets. Because of this, red-orange–fluorescent dyes should not be used in conjunction with the SYTO RNASelect dye.

Protocols

Labeling Live Eukaryotic Cells

Below is a general procedure for labeling live, cultured cells that are adhering to coverslips. The protocol was developed on and optimized for bovine pulmonary artery endothelial (BPAE) cells, and serves as a general guideline for staining other cell types.

1.1 Prepare the labeling solution. The labeling solution will consist of a 500 nM solution of RNASelect green fluorescent cell stain in cell-culture medium appropriate for the cells being stained, or phosphate-buffered saline (PBS). To prepare 1 mL of the labeling solution, first make a 5 µM intermediate stock by adding 1 µL of the 5 mM stock solution to 1.0 mL of the medium, mixing and then adding 100 µL of the 5 µM intermediate stock to 900 µL of the medium. This 500 nM labeling solution should be pre-warmed at 37°C prior to application and used immediately. If the solution is not used within a few minutes of preparation, the dye may begin to precipitate and cause undesirable debris on the coverslip.

1.2 Label the cells. Apply a sufficient amount of the prewarmed 500 nM labeling solution to cover the cells adhering to a coverslip. Incubate for 20 minutes at 37°C.

1.3 Rinse the cells. When labeling is complete, remove the solution; rinse the cells twice in cell-culture medium or PBS; and rest the cells for 5 minutes in medium at 37°C. The cells on the coverslip should then be ready for imaging.

1.4 Fix the cells in methanol (optional). If desired, the labeled cells can be fixed with pre-chilled methanol for 10 minutes at –20°C, followed by several washes in PBS. With methanol fixation, the labeling pattern should be the same as with unfixed cells, except that the punctate mitochondrial signal disappears upon fixation. Formaldehyde should not be used, as it redistributes the SYTO RNASelect stain, increasing the cytoplasmic and nuclear background.

Labeling Fixed Eukaryotic Cells

The following general procedure for labeling methanol-fixed, cultured cells adhering to coverslips was optimized for BPAE cells, and the method serves as a general guideline for staining other cell types

2.1 Fix the cells. Remove the coverslips from the culture medium, and fix in pre-chilled methanol at –20°C for 10 minutes. With methanol fixation, the labeling pattern should be the same as with live cells, except that the punctate mitochondrial signal will be absent. Formaldehyde should not be used.

2.2 Wash the cells. Wash the cells for 5 minutes in PBS. Repeat the wash two times.

2.3 Prepare the labeling solution. The labeling solution will consist of a 500 nM solution of RNASelect green fluorescent cell stain in PBS. To prepare 1 mL of labeling solution, first make a 5 µM intermediate stock by adding 1 µL of the 5 mM stock solution to 1.0 mL of PBS, mixing and then adding 100 µL of the 5 µM intermediate stock to 900 µL of PBS. This 500 nM staining solution should be used immediately. If the solution is not used within a few minutes of preparation, the dye may begin to precipitate and cause undesirable debris on the coverslip.

2.4 Label the cells. Apply the labeling solution (prepared in step 2.3). Incubate for 20 minutes at room temperature.

2.5 Wash the cells. Wash the cells for 5 minutes in PBS. Repeat the wash two times.

2.6 Prepare the cells for viewing. Counterstain the cells as desired, and mount in ProLong® antifade mounting medium for optimal viewing.

Staining Bacterial Cells

Bacterial cells can also be stained with the RNASelect green fluorescent cell stain. Working concentrations of the dye should be kept below 1 µM. The 500 nM labeling solution, as prepared in step 2.3, is a good starting point for determining the optimal staining concentration.

Reference

1. Alberts, B., et al. Molecular Biology of the Cell, 3rd ed. Garland Publishing, Inc. (1994).

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

Cat #	Product Name	Unit Size
S32703	SYTO® RNASelect™ green fluorescent cell stain *5 mM solution in DMSO*	100 µL

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

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