Cell Navigator™ Live Cell RNA Imaging Kit *Green Fluorescence*

Catalog number: 22630
Unit size: 100 Tests

Component	Storage	Amount
Component A: StrandBrite™ RNA Green	Freeze (<-15 °C)	25 µL (400X in DMSO)
Component B: Live Cell Staining Buffer	Freeze (<-15 °C), Minimize light exposure	20 mL

OVERVIEW

Detecting and imaging RNA molecules in living cells is extremely important for a wide variety of molecular biology procedures including physical transportation, interpretation of genetic information, regulation of gene expression and some essential bio-catalytic roles. The major challenge to stain RNA in living cells is the interferences caused by DNA. In order to address the difficulty, a novel green fluorogenic dye was developed as a RNA-selective probe. AAT Bioquest's Cell Navigator™ Live Cell RNA Imaging Kit includes StrandBrite™ RNA Green as it specifically binds RNA in cells. Compared to commercial SYTO® RNA Select dye for RNA staining in vivo, StrandBrite™ RNA Green shows brighter signal and much better selectivity to RNA. In addition, this kit can stain RNA in both living cells and fixed cells.

AT A GLANCE

Protocol summary

1. Prepare cells
2. Add StrandBrite™ RNA Green working solution
3. Incubate for 30 - 60 minutes
4. Analyze the cells under fluorescence microscope at Ex/Em = 490/520 nm (FITC filter set)

Important Thaw all the components at room temperature before starting the experiment.

KEY PARAMETERS

Instrument:	Fluorescence microscope
Excitation:	490 nm
Emission:	520 nm
Instrument specification(s):	FITC filter set
Recommended plate:	Black wall/clear bottom

PREPARATION OF CELL SAMPLES

For guidelines on cell sample preparation, please visit
https://www.aatbio.com/resources/guides/cell-sample-preparation.html

SAMPLE EXPERIMENTAL PROTOCOL

1. Culture cells to a density optimum for imaging according to your specific induction protocol (about 1 - 2 × 10^4 cells/well/96-well plate).

2. **For living cells:** Incubate cells with StrandBrite™ RNA Green (Component A) diluted 400X in medium or live cell staining buffer (Component B) at room temperature for 30 - 60 minutes (100 µL/well).

   Note 25 µL of StrandBrite™ RNA Green (Component A) is enough for one 96-well plate. Protect from light and avoid repeated freeze-thaw cycles. The appropriate incubation time depends on the individual cell type and cell concentration used. Optimize the incubation time for each experiment. See figure 1 for details.

3. **For fixed cells:** Fix cells with pure methanol for 1 minute at room temperature, then wash with PBS. Immerse cells in 1% Triton-100 for 2 minutes, then wash with PBS twice. Incubate cells with StrandBrite™ RNA Green (Component A) at the concentration of 1X in PBS at room temperature for 15 - 30 minutes.

   Note It is recommended to increase either the labeling concentration or the incubation time to allow the dye to accumulate if the cells do not appear to be sufficiently stained. See figure 1 for details.

4. (Optional) Wash the cells with PBS for 1 - 2 times, add 100 µL PBS to each well.

5. Monitor fluorescence intensity with fluorescence microscope at Ex/Em = 490/520 nm (FITC channel).

EXAMPLE DATA ANALYSIS AND FIGURES

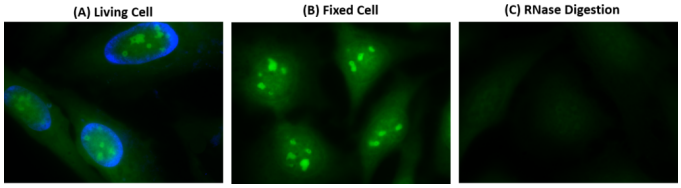

Figure 1. Fluorescence images of RNA staining in HeLa cells. (A) Live cells were stained using Cell Navigator™ Live Cell RNA Imaging Kit (Green, Cat#22630) and counter-stained with Hoechst 33342 (Blue, Cat#17530). (B) Cells fixed in methanol were stained using the same kit. (C) After staining, fixed HeLa cells were incubated with 0.5 mg/mL RNase at 37 ºC for 1 hour. Image of RNase digest test indicates the high selectivity of StrandBrite™ RNA Green. The green fluorescence signal were measured using a fluorescence microscope with a FITC filter.

DISCLAIMER