Selective Analysis of RNA in Live and Fixed Cells with StrandBrite RNA Green

Zhen Luo, Jixiang Liu, Yunting Xi, Qin Zhao, Ruogu Peng, Jinfang Liao, Zhenjun Diwu
AAT Bioquest, Inc., 520 Mercury Drive, Sunnyvale, CA 94085

Introduction

Flow cytometry analysis can provide important spatial and temporal information of multiple biomarkers in individual cells simultaneously. However, most of the flow cytometry analysis are still limited to total DNA and protein expression in cells. It is well known that detecting and imaging RNA molecules in live cells is extremely important for a wide variety of molecular biology applications. To measure RNA level in cells, commonly used techniques such as polymerase chain reaction (PCR), microarrays, and fluorescence in situ hybridization (FISH) are still limited to lysed and fixed cells. The major challenge to analyze RNA in live cells is the interferences caused by DNA. To address these difficulties, we have developed StrandBrite RNA Green, an excellent RNA-selective probe that generates significantly enhanced green fluorescence upon binding to RNA. It has been successfully used for flow cytometric analysis of live cells.

Material and Methods

Cell culture:
HeLa cells were seeded overnight in a 96-well black wall/clear bottom costar plate at 37 ºC. Non-adherent cell line Human T lymphocyte Jurkat cells were grown at 37 ºC in RPMI 1640.

RNA staining in live and fixed cells:
Live cells were incubated with StrandBrite RNA Green in medium for 30-60 minutes. For fixed cell staining, cells were fixed with 4% formaldehyde in PBS for 30 minutes and then stained with StrandBrite RNA Green in PBS for 30 minutes.

RNase and DNase digest test in cells:
Cells were first fixed in methanol for 1 minute then permeabilized by immersing the cells in 1% Triton X-100 for 2 minutes. After rinsing with PBS, cells were stained using StrandBrite RNA Green for 15 minutes. After removing the unbound dye outside cells, DNase and RNase were added and incubated with cells at 37 ºC for 30 minutes.

RNA synthesis inhibition test:
Jurkat cells were first incubated with antinomycin D (0-10 µg/mL) at 37 ºC for 30 minutes, and re-incubated in the full medium for 3-6 hours. After washing 3 times with HBSS buffer, cells were labeled with the StrandBrite RNA Green for 15 minutes before analyzed by flow cytometer (ACEA NovoCyte 3000).

Imaging RNA in Live and Fixed Cells

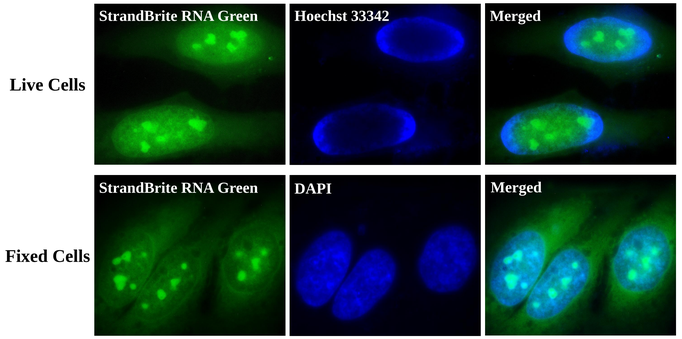

Figure 1. Fluorescence images of live and fixed HeLa cells stained with StrandBrite RNA Green (Green) and counter-stained with Hoechst 33342 or DAPI (Blue). Fluorescence signal were measured using a fluorescence microscope with FITC filter.

StrandBrite RNA Green vs. SYTO® RNASelect

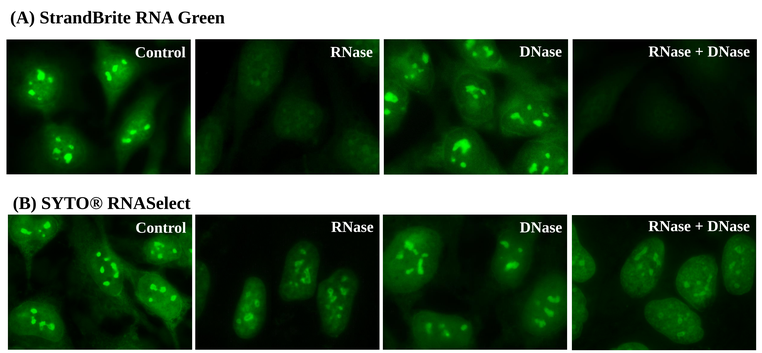

Figure 2. RNase and DNase digest test of HeLa cells stained with (A) StrandBrite RNA Green or (B) SYTO RNASelect, respectively. Both RNA probes were tested in the concentration of 1.5 µM. DNase of 50 U/mL and RNase of 50 µg/mL were added into cells. Fluorescence images were taken using a fluorescence microscopy with FITC filter.

Flow Cytometric Analysis of RNA in Cells

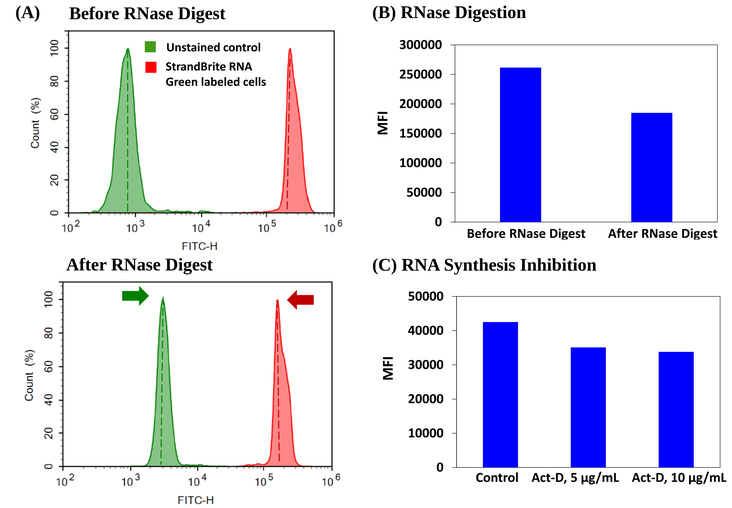

Figure 3. (A) Flow cytometric analysis of RNase digest in Jurkat cells stained with StrandBrite RNA Green. (B) Changes in mean fluorescence intensity (MFI) of Jurkat cells before and after RNase digest. The MFI in unstained cells was used as control and subtracted from the MFI of StrandBrite RNA Green labeled cells. (C) Changes in MFI of live Jurkat cells stained with StrandBrite RNA Green upon Actinomycin-D treatment. Fluorescence intensity was measured using ACEA NovoCyte flow cytometer.

Selectivity Test of StrandBrite RNA Green

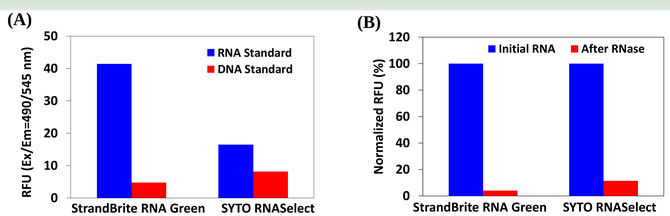

Figure 4. Comparison of StrandBrite RNA Green and SYTO RNASelect in (A) RNA selectivity assay and (B) RNase digest test. Both RNA probes were tested in the same concentration and with 5 µg/mL of RNA or DNA standards. Relative fluorescence unit (RFU) was measured using a Gemini fluorescence microplate reader.