



EZClick™ Global RNA Synthesis Assay Kit (FACS/Microscopy), Red rev 05/20

(Catalog # K718-100; 100 assays; Store at -20°C)

I. Introduction:

RNA plays crucial role in coding, decoding and regulation of genes, and protein expression in all living cells. The ability to detect newly synthesized RNA or changes in RNA levels under various physiological conditions, or resulting from disease, environmental damage, or drug treatments is an important aspect of toxicological profiling. Many anti-cancer drugs inhibit transcription, and most transcription inhibitors have useful pharmacological properties. **Biovision's EZClick™ Global RNA Synthesis Assay Kit** provides a simple and robust tool for detection of global RNA transcription temporally and spatially or changes in RNA levels directly in living cells. *De novo* synthesized RNA can be detected with a simple procedure without the use of radiolabeling or antibodies. Our approach relies on the incorporation of cell permeable 5-EU (Ethylnyl uridine) into nascent RNA, but not into DNA, instead of its natural uridine analog. 5-EU can be used as a replacement for BrU (5-Bromo-uridine) to measure *de novo* synthesized RNA in proliferating cells. Modified RNA is detected by click chemistry with azide-containing dye that enables for multiplex analyses with other probes, or detection of RNA-interactive proteins for deeper biological insights. Our kit provides sufficient materials for 100 assays for analysis by FACS or detection by fluorescence microscope. We include Actinomycin D, an inhibitor RNA synthesis that serves as an experimental control.

II. Applications:

- Detection and quantification of RNA transcription *in vitro* and *in vivo* by fluorescence microscope and/or FACS.
- Screening for genotoxic compounds and effectors of RNA biosynthesis in proliferating cells.
- Evaluating effects of anti-cancer drugs and genotoxic agents.

III. Sample Type:

- Suspension or adherent cell cultures

IV. Kit Contents:

Components	K718-100	Cap Code	Part Number
EZClick™ Wash Buffer (10X)	25 ml	NM	K718-100-1
Fixative Solution	10 ml	WM	K718-100-2
Permeabilization Buffer (10X)	25 ml	NM/Blue	K718-100-3
EZClick™ RNA Label (100X)	100 µl	Clear	K718-100-4
Copper Reagent (100X)	100 µl	Blue	K718-100-5
EZClick™ Fluorescent Azide (100X)	100 µl	Red	K718-100-6
Reducing Agent (20X)	500 µl	Yellow	K718-100-7
DAPI (1000X)	20 µl	Blue/Amber Vial	K718-100-8
Actinomycin D (100X)	10 µl	Purple	K718-100-9

V. User Supplied Reagents and Equipment:

- Tissue culture vessels and appropriate culturing media; flow cytometry vessels
- Phosphate Buffered Saline (PBS, pH 7.4)
- Sterile 0.1% Gelatin Solution (optional, only required for adhering suspension cells to the surface)
- Flow cytometer equipped with laser capable of excitation at 488 and 530/590 nm emission filters respectively
- Fluorescence microscope capable of excitation and emission at 440/490 and 540/580 nm respectively

VI. Storage Conditions and Reagent Preparation:

Upon arrival, store the entire kit at -20°C protected from light. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay.

- **EZClick™ Wash Buffer (10X) and Permeabilization Buffer (10X):** Thaw at 37°C to dissolve completely. Dilute the 10X stock at 1:10 in sterile water, mix well. Store at 4°C.
- **Fixative Solution: Divide into aliquots and store at -20°C, protected from light.**
- **Remaining components:** Store at -20°C protected from light. While in use, keep on ice and minimize light exposure.

VII. Assay Protocol:

Notes:

This assay was developed with HeLa (adherent) and Jurkat (suspension) cells and can be modified for any cell line. The protocol below refers to a 96-well tissue culture plate; adjust volumes accordingly for other plate formats. The assay volume is 100 µl. Growth conditions, cell number per well and other factors may affect the incorporation rate of the RNA Label; therefore optimize the assay for your cell type. We suggest an initial test of several EZClick™ RNA Label concentrations to find best conditions for tested cell type and experimental design. Avoid stressing the cells by washes or temperature changes prior to incubation with EZClick™ RNA Label. All steps should be carried out at room temperature (RT) unless otherwise specified; equilibrated all buffers to RT prior to the experiment.

1. Labeling of control and experimental cells:

- Obtain cell suspension of desired density and seed directly into tissue culture vessels, or on coverslips for high resolution microscopy. **To immobilize suspension cells for microscopy:** add 100 µl of 0.1% gelatin solution directly into the wells, tilt the plate to cover the entire well surface and place it in a tissue culture hood for 1 hour. Gently remove the gelatin solution and seed your cells. Allow the cells to recover overnight before the treatment.
- Next day, treat the cells with appropriate effectors according to your protocol; do not add treatment to the positive and negative control cells. **Negative Control** (cells not exposed to the RNA Label or treatment), **Positive Control** (cells incubated with 1X RNA Label only). To use included Actinomycin D as an inhibitor of RNA synthesis, dilute it 1:100 directly into the culture medium and incubate the cells for 4 hours at 37°C.
- Dilute EZClick™ RNA Label (100X) to 1X final concentration directly into the experimental, positive control, and Actinomycin D-treated cells respectively. *Do not add the EZClick™ RNA Label into the **Negative Control** cells.* To avoid potential reversibility of drug action on RNA synthesis, do not remove the drug-containing media during incubation with 1X RNA Label. Incubate the cells for additional hour in a 37°C incubator, or for the period of time required by your experimental protocol.

