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ab228561 RNA Synthesis Assay Kit

For the measurement of global RNA transcription temporally and spatially or changes in RNA levels directly in living cells.

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Table of Contents

1. Overview

RNA Synthesis Assay Kit (ab228561) 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. The approach relies on the incorporation of cell permeable 5-EU (Ethynyl 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. The 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.

Prepare cell samples.

Add RNA Label to 1X final concentration.

Remove culture medium and rinse the cells once with PBS.

Fix cells

Permeabilize cells

Add RNA Reaction cocktail. Incubate for 30 min at room temperature. Protect from light. Remove the reaction cocktail and wash cells three times in Wash Buffer.

Stain DNA with DAPI (Optional)

Analyze by Fluorescence Microscope Imaging or FACS analysis.

2. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Aliquot components in working volumes before storing at the recommended temperature.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
10X Wash Buffer IV/Wash Buffer (10X)	25 mL	-20°C	4°C
Fixative Solution I/Fixative Solution	10 mL	-20°C	-20°C
10X Permeabilization Buffer/Permeabilization Buffer (10X)	25 mL	-20°C	4°C
100X RNA Label/RNA Label (100X)	100 µL	-20°C	-20°C
100X Copper Reagent/Copper Reagent (100X)	100 µL	-20°C	-20°C
100X Fluorescent Azide I/Fluorescent Azide (100X)	100 µL	-20°C	-20°C
20X Reducing Agent/Reducing Agent (20X)	500 µL	-20°C	-20°C
1000X DAPI/DAPI (1000X)	20 µL	-20°C	-20°C
100X Actinomycin D/Actinomycin D (100X)	10 µL	-20°C	-20°C

3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- 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

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

Read entire protocol before performing the assay.

5.1 10X Wash Buffer IV/Wash Buffer (10X) (25 mL)

Thaw at 37 °C to dissolve completely. Dilute the 10X stock 1:10 in sterile water, mix well. Store at 4 °C.

5.2 Fixative Solution I/Fixative Solution (10 mL)

Ready to use as supplied. After opening, divide into aliquots and store at -20 °C, protected from light.

5.3 10X Permeabilization Buffer/Permeabilization Buffer (10X) (25 mL)

Thaw at 37 °C to dissolve completely. Dilute the 10X stock 1:10 in sterile water, mix well. Store at 4 °C.

5.4 100X RNA Label/RNA Label (100X) (100 µL)

Ready to use as supplied. While in use, keep on ice and minimize light exposure.

5.5 100X Copper Reagent/Copper Reagent (100X) (100 µL)

Ready to use as supplied. While in use, keep on ice and minimize light exposure.

5.6 100X Fluorescent Azide/Fluorescent Azide (100X) (100 µL)

Ready to use as supplied. While in use, keep on ice and minimize light exposure.

5.7 20X Reducing Agent/Reducing Agent (20X) (500 µL)

Ready to use as supplied. While in use, keep on ice and minimize light exposure.

5.8 1000X DAPI/DAPI (1000X) (20 µL)

Ready to use as supplied. While in use, keep on ice and minimize light exposure.

5.9 100X Actinomycin D/Actinomycin D (100X) (10 µL)

Ready to use as supplied. While in use, keep on ice and minimize light exposure.

6. Assay Procedure

– Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.

6.1 Labeling of control and experimental cells:

1. 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.

2. 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.

3. Dilute 100X RNA Label/RNA Label (100X) to 1X final concentration directly into the experimental, positive control, and Actinomycin D-treated cells respectively. Do not add the 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.

4. Terminate the experiment by removal of the culture medium and rinse the cells once with 100 µL of PBS, discard the supernatant. For immobilized suspension cells: Centrifuge the plate at 300 x g (or the lowest centrifuge setting) for 5 minutes to deposit the cells onto the surface. Tilt the plate and gently remove the media with a pipette tip. It is important to avoid excessive centrifugation speeds, which can damage the cells. Make note of the place that is used and perform subsequent

aspirations from the same place. Proceed to the Fixation and Permeabilization. Pellet the suspension cells at 300 x g for 5 min throughout the entire protocol!

6.2 Fixation and Permeabilization:

1. For adherent cells: Add 100 µL of Fixative Solution I/Fixative Solution to each well and incubate the cells for 15 minutes at room temperature protected from light. Remove the fixative and wash the cells once with 200 µL of 1X Wash Buffer IV/Wash Buffer, remove the wash. Add 100 µL of 1X Permeabilization Buffer. Incubate the cells for 10 minutes at room temperature and remove the Permeabilization Buffer.
2. For suspension cells: Re-suspend the cells in 100 µL of Fixative Solution I/Fixative Solution and incubate for 15 minutes at room temperature protected from light. Centrifuge cells at 900 x g for 5 minutes and remove the fixative. Wash the cells once with 0.5 mL of 1X Wash Buffer IV/Wash Buffer. Centrifuge cells at 900 x g for 5 minutes and remove the supernatant. Re-suspend the cells in 100 µL of 1X Permeabilization Buffer. Incubate the cells for 10 minutes at room temperature. Centrifuge cells at 900 x g for 5 minutes and remove the Permeabilization Buffer. Proceed to RNA reaction and total DNA staining.

6.3 RNA reaction and total DNA staining:

1. Reaction cocktail: Prepare 1X reaction cocktail according to the table below. Volumes should be multiplied by number of samples and reagents added in the exact order. Use the reaction cocktail within 15 minutes of preparation.

Δ Note: Cells should be protected from light during and following the reaction and DNA staining.

Component	Reaction Mix (µL)
PBS	93 µL
100X Copper Reagent/Copper Reagent (100X)	1 µL
100X Fluorescent	1 µL

Azide/Fluorescent Azide (100X)	
20X Reducing Agent/Reducing Agent (20X)	5 µL

2. RNA Reaction: Add 100 µL of 1X Reaction cocktail to each sample and incubate the cells for 30 minutes at room temperature protected from light. Centrifuge cells at 900 x g for 5 minutes and remove the reaction cocktail. Wash cells twice in 200 µL of Wash Buffer IV/Wash Buffer and centrifuge cells at 900 x g for 5 minutes after each wash. Remove the Wash Buffer IV/Wash Buffer and resuspend the cells in 100 µL of PBS. Proceed to DNA staining. If no DNA staining is desired, proceed to Microscopic or FACS analysis.
3. DNA staining: Prepare 1X dilution of DAPI Stain and add 100 µL per well. Incubate the cells for 20 minutes at room temperature or refrigerate at 4 °C protected from light. Centrifuge cells at 900 x g for 5 minutes and remove the DNA stain solution. Wash the cells once with 100 µL of PBS.

Δ Note: cells are compatible with all methods of slide preparation including wet mount or prepared mounting media.

6.4 Analysis:

1. Fluorescence Microscope Imaging: Analyze samples for red fluorescence generated by labeled RNA and nuclear DNA under the UV filter respectively.
2. FACS analysis: Harvest the cells by preferred method and wash with 0.5 mL of ice-cold PBS. Centrifuge cells at 900 x g for 5 minutes and re-suspend the pellets with 100 µL of ice-cold PBS. Transfer the cell suspension into flow cytometry vessels. Analyze samples in FL-2 channel for signal generated by RNA during click reaction.

Δ Note: Trypsin can be used to collect the adherent cells prior to FACS analysis.

7. Typical Data

Data provided **for demonstration purposes only.**

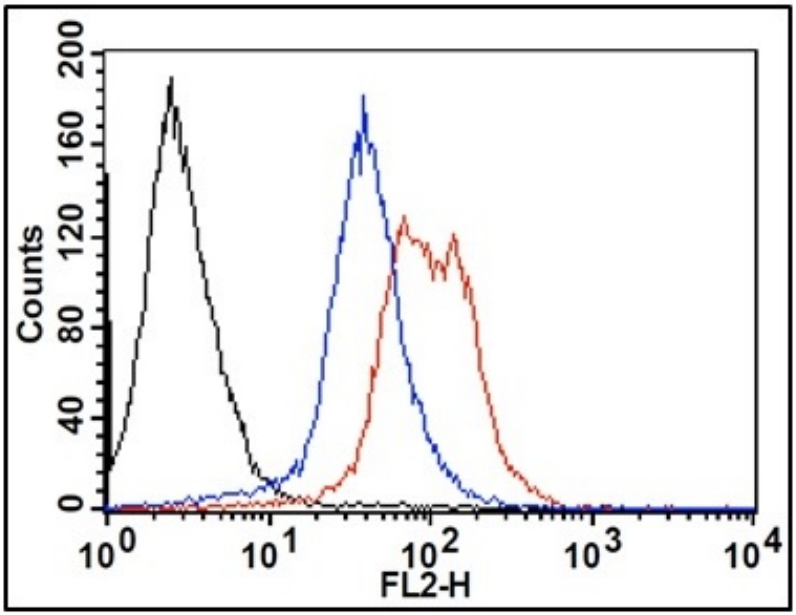

Figure 1. Jurkat cells (1X10^6 cells/mL) were pre-treated with vehicle (black line) or 1 X Actinomycin D (blue line) for 4 hours at 37°C prior to 1 hour incubation with RNA Label (red line). Cells were then processed for detection of *de novo* synthesized RNA according to the included protocol. Fluorescence measured in FL-2 channel clearly shows the inhibitory effect of Actinomycin D on RNA synthesis.

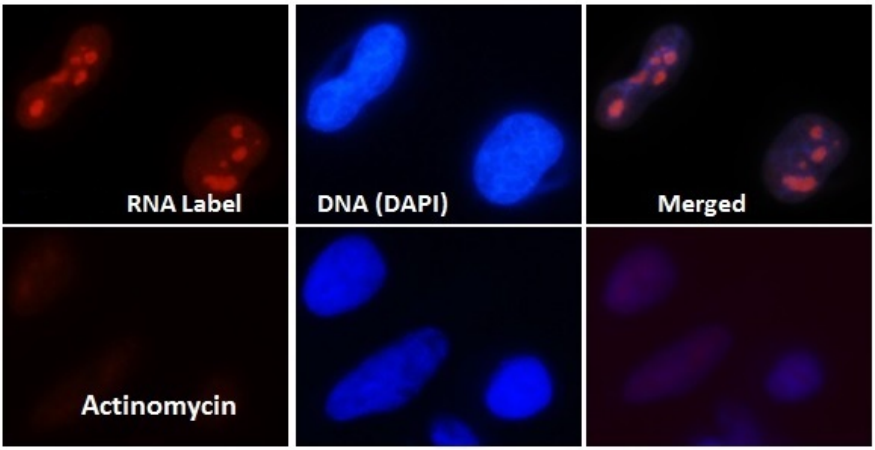

Figure 2. HeLa cells (10^5 cells/ mL) were pre-treated either with vehicle (top) or Actinomycin D (bottom) for 4 hours at 37°C prior to 1 hour incubation with RNA Label. *De novo* synthesized RNA was detected by Fluorescence Microscope. Reduced red fluorescence in panel B confirms the inhibitory effect of Actinomycin D on RNA biosynthesis. Nuclear staining in both panels confirms that red fluorescence is the result of RNA Label incorporation.

8. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)