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ab273285 Global RNA Synthesis Assay Kit (Green)

View Kit datasheet: https://www.abcam.com/ab273285
(use https://www.abcam.cn/ab273285 for china, or
https://www.abcam.co.jp/ab273285 for Japan)

For the determination of RNA Synthesis activity in adherent and suspension 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.

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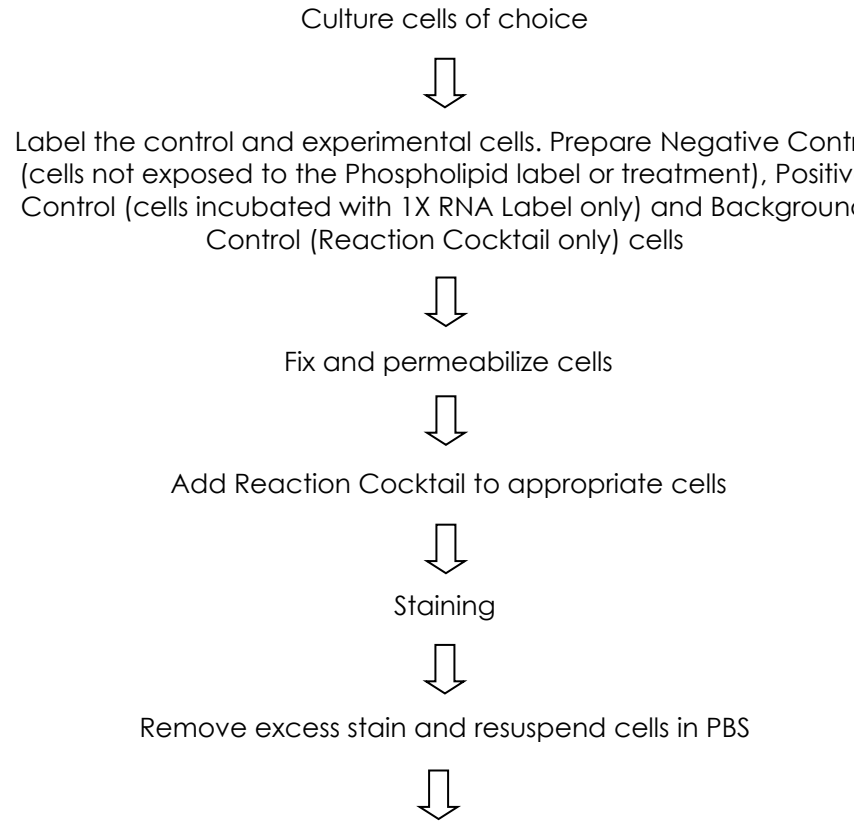
1. Overview

Global RNA Synthesis Assay Kit (Green) (ab273285) 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 using an azide-containing dye that enables for multiplex analyses with other probes, or detection of RNA-interactive proteins for deeper biological insights.

The kit includes Actinomycin D, an inhibitor of RNA synthesis that serves as an experimental control.

2. Protocol Summary



3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Reagents Preparation section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

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

7. 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
- Phosphate Buffered Saline (PBS, pH 7.4)
- A 6-, 12-, 24-, or 96-well clear plates should be used only for cell culturing. The measurement of fluorescence should be performed in white opaque plates. Alternatively, sterile opaque plates can be used for both, culturing and measurements
- Multi-well spectrophotometer and Fluorescence microscope (optional) capable of measuring Ex 494 and Em 521 nm spectra.

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.
- Hot plate/dry heat block or microplate incubator

9. Reagent Preparation

Briefly centrifuge small vials prior to opening.

9.1 10X Wash Buffer IV/10X Wash Buffer:

Dilute the 10X stocks 1:10 in sterile water, mix well. Store at 4°C.

9.2 10X Permeabilization Buffer:

Dilute the 10X stocks 1:10 in sterile water, mix well. Store at 4°C.

9.3 Fixative Solution I/Fixative Solution:

Divide into aliquots and store at -20°C, protected from light.

All other components should be stored at -20°C protected from light. While in use, keep on ice and minimize light exposure.

10. Assay Protocol

- This assay was developed with Jurkat (suspension) and HeLa (adherent) cells and can be modified for any cell line.
- The following protocol has been optimized for a 96-well opaque plate at 1X106 cells per well, using fewer cells per well or clear plate will result in decreased signal.
- We suggest testing growth conditions, cell number per well and several concentrations of the RNA Label, to find the best experimental design for your cell type.
- The assay volume is 100 µl; adjust volumes accordingly for other plate formats.
- Avoid stressing the cells by washes or temperature changes prior to incubation with RNA Label.
- All steps should be carried out at room temperature (RT) unless otherwise specified; equilibrate all buffers to RT prior to the experiment.

Labeling of control and experimental cells

10.1 Plate suspension or adherent cells at a desired density and allow for overnight recovery before treatment. Ensure that adherent cells are sub-confluent.

10.2 Include appropriate controls and account for cell loss during the processing. Negative control (cells not exposed to the Phospholipid Label or treatment), Background control (cells treated with Reaction Cocktail only), Positive control (cells incubated with 1X RNA Label only).

10.3 Next day, remove the media and treat the cells with appropriate effectors according to your protocol; do not add treatment to the positive and negative control cells.

10.4 For suspension cells: Centrifuge the plate at 500 *x g* (or the lowest centrifuge setting) for 5 mins at RT to pellet the cells.

10.5 Tilt the plate and gently remove the media with a pipette tip.

Δ Note: Avoid excessive centrifugation speeds, which can damage the cells. Use these centrifugation settings throughout the entire protocol.

10.6 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 hrs at 37°C.

10.7 Dilute 100X RNA Label/RNA Label (100X) to 1X final concentration with culture medium and add into the Experimental, Positive control and Actinomycin D-treated cells respectively.

10.8 Incubate the cells for additional 1-24 hrs, or time required by your experimental protocol in a 37°C incubator. Do not add the RNA Label into the Negative control cells. Do not remove the drug containing media during incubation with 1X RNA Label to avoid potential reversibility of drug action on label incorporation.

10.9 Harvest the suspension cells by centrifugation. Optionally, detach adherent cells (e.g. trypsinize and quench with media), and harvest by centrifugation.

10.10 Wash the cells once with 100 µl of PBS, discard the supernatant and proceed to the Fixation and Permeabilization.

Fixation and Permeabilization

10.11 For adherent and suspension cells: Add 100 µl of Fixative Solution I/Fixative Solution to each well and incubate the cells for 15 mins at RT protected from light.

10.12 Remove the Fixative Solution I/fixative and wash the cells once with 100 µl of 1X Wash Buffer IV/Wash Buffer, remove the wash.

10.13 Add 100 µl of 1XPermeabilization Buffer and incubate the cells for 10 mins at RT.

10.14 Remove the Permeabilization Buffer and replace it with a 20 µl of fresh aliquot. Proceed to RNA reaction.

RNA reaction:

- Volumes should be multiplied by number of samples and reagents and added in the exact order.
- Use the reaction cocktail within 15 mins of preparation.
- Cells should be protected from light during and following the reaction and DNA staining.

10.15 Prepare 1X Reaction Cocktail according to the table below.

Component	Amount per reaction
PBS	93 µL
100X Copper Reagent/Copper Reagent (100X)	1 µL
Fluorescent Azide II/Fluorescent Azide (100X)	1 µL
20X Reducing Agent/Reducing Agent (20X)	5 µL

10.16 Add 100 µl of 1X Reaction cocktail to each sample and incubate the cells for 30 mins at RT protected from light.

10.17 Remove the reaction cocktail and wash cells three times in 100 µl of Wash Buffer IV/Wash Buffer.

10.18 Suspend the cells in 100 µl of Wash Buffer IV/Wash Buffer.

10.19 For detection cells must be analyzed immediately in the plate reader at Ex/Em= 494/521 nm in end point mode to determine change in fluorescence of compounds and controls after background subtraction or imaged with fluorescence microscope directly in the plate following DNA staining. Analyze samples for green fluorescence generated by *de novo* synthesized RNA.

10.20 For the Azide Fluorescence Curve: To increase the accuracy of your data, instantly prepare azide fluorescence curve for each condition and cell line using cells previously detected for green fluorescence (Step 10.19).

10.21 In a 96-well white opaque plate, prepare a series of dilutions of your cell suspension starting with the same volume and number of cells as in the experimental wells. Dilute the cells by factor of 2, prepare at least 6 dilutions. To minimize the error,

aliquot at least 2 wells per dilution. Measure fluorescence and calculate average for each dilution, subtract the background value. Plot the Azide Fluorescence Curve to obtain fluorescence per cell number and the detection limit for your assay. Optional: standard curve of Fluorescent Azide II/Fluorescent Azide concentration per well can be prepared in the same manner to obtain the least detectable amount of azide for your experiment.

11. Typical Data

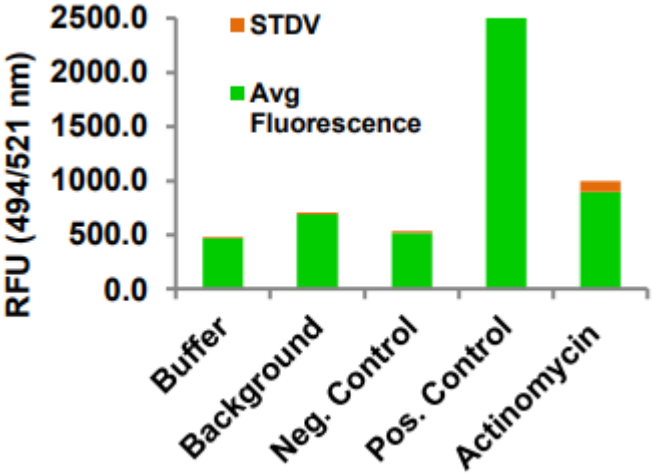


Figure1: Plate reader analyses on Jurkat cells (1X10^6 cells/well) with controls and Actinomycin treatment. Average fluorescence standard deviation plotted for 3 replicates per condition.

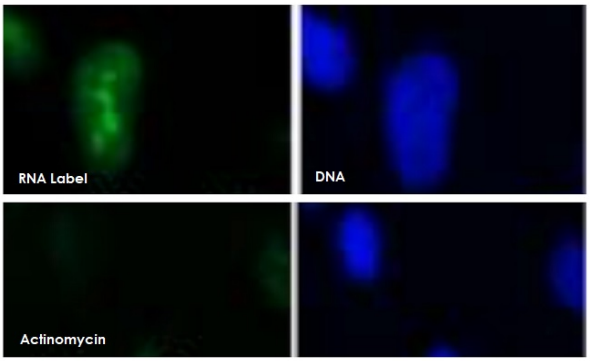


Figure 2: Metabolic labeling of RNA on proliferating HeLa cells. 1 x 10^5 cells were pre-treated with vehicle or 1 X Actinomycin D for 4h at 37°C prior to 24 hours incubation with RNA Label then processed for detection of *de novo* synthesized RNA according: Upper panel corresponds to green fluorescence of *de novo* synthesized RNA. The lower panel shows cells treated with Actinomycin. Nuclear staining confirms that the green signal results from RNA Label incorporation.

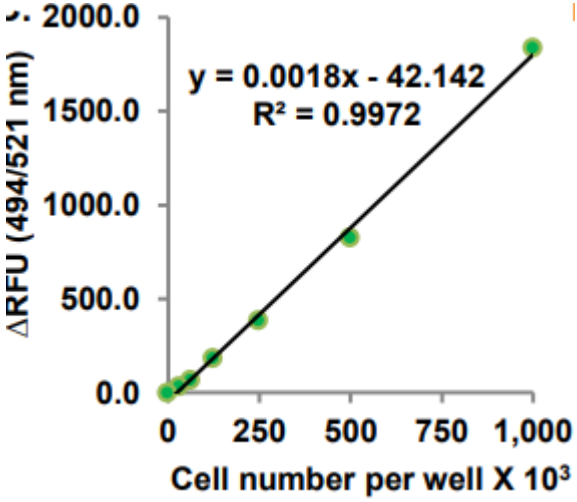


Figure 3. Azide Fluorescence Curve of Jurkat cells prepared for this assay. Detection limit corresponds to about 31,250 of Jurkat cells per well. A new curve must be obtained for each experiment and the cell line.

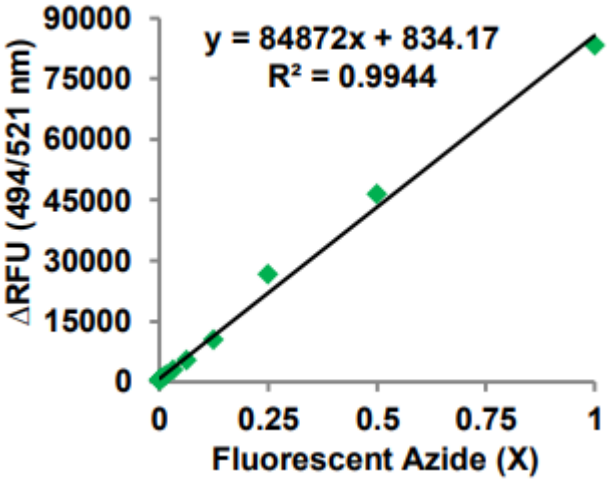


Figure 4. Azide Fluorescence Curve in 0-1 X range. This is reference data and it should not be used to interpret actual results. Your data will depend on the cell type and tested compound.

12. FAQ / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

13. 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)