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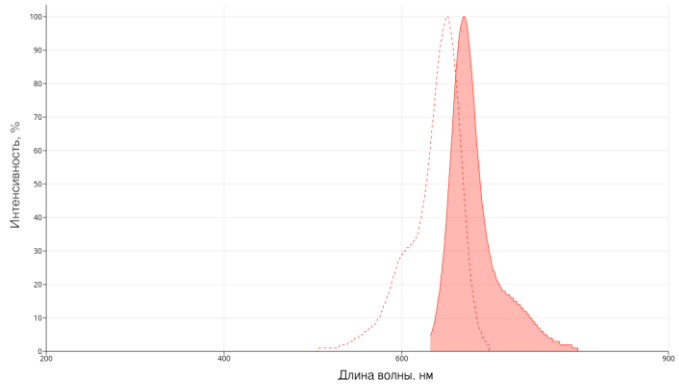
Cy5 RNA labeling kit

Catalog number: LBL-RNA-3-1, LBL-RNA-10-1

Description:

The kit is intended for introducing the Cyanine 5 (Cy5) fluorescent label at the 3'-OH group of an RNA molecule. The absorption and emission spectrum of the Cy5 fluorescent dye is shown in the figure below.



Type	wave, nm
Excitation	651
Emission	670

Content:

	LBL-RNA-3-1 3 RNA samples	LBL-RNA-10-1 10 RNA samples
Cy5 dye solution	15 ul	50 ul
Oxidation buffer	100 ul	200 ul
Cy5 reaction buffer	100 ul	200 ul
Oxidizer	100 ul	300 ul
Precipitation buffer	100 ul	200 ul
DEPC water	2x1500 ul	3x1500 ul

Reagents and equipment necessary for work

- Centrifuge capable of reaching speeds of at least 16,000 rcf
- Analytical balances with an accuracy of 1 mg
- Polyethylene microcentrifuge tubes 0.5 – 2 ml
- Set of automatic dispensers
- Ethyl alcohol 96%

Before starting work

Preparation of an oxidizing solution:

- measure 10 - 30 mg of oxidizing agent into a 1.5 ml test tube on a scale. Then add DEPC-treated water to the test tube with the oxidizing agent.

Calculate the amount of water using the formula:

$$Volume, ml = \frac{Oxidizer, mg}{85}$$

- Stir the contents of the test tube until the oxidizing crystals are completely dissolved. The oxidizing solution can be stored at room temperature for no more than 7 days from the date of preparation. It is recommended to store this buffer at room temperature, because At lower temperatures, precipitation occurs.

Cool 96% ethyl alcohol from -19 to -25°C.

Preparation of 1000 µl wash solution:

- add 800 µl of ethyl alcohol 96% and 200 µl of DEPC-treated water to a 1.5 ml test tube and mix.

Protocol

1. RNA oxidation.

- Add 10 µl of buffer for the oxidation reaction to a test tube with a capacity of 0.5 – 1.5 ml.
- Add 1 - 20 µl of sample containing 5 - 150 µg of RNA.
- Add 5 µl of oxidizing agent solution.
- Dilute the reaction mixture with DEPC-treated water to 50 µl.

- Mix the contents of the tube and incubate for 1 hour in a dark place at room temperature 20 – 30 °C.

2. Intermediate RNA purification.

- Add 5 µl of precipitation buffer and 150 µl of 96% ethanol, pre-cooled to -19°C, to each tube. Mix by shaking.
- Incubate samples at temperatures from -19°C to -25°C for 15-30 minutes.
- Centrifuge samples for 20 – 30 minutes at 16000 rcf and temperature 4°C.
- Carefully remove the supernatant using a dispenser and add 200 µl of washing solution. Incubate with the solution for 0.5 - 1 minute at room temperature.
- Remove the washing solution. Leave the tubes with the RNA precipitate with the lids open in a dark place for 15 - 20 minutes.

3. RNA labeling.

Add 35 µl of DEPC-treated water to the test tube with the RNA precipitate. Dissolve the precipitate by stirring using a dispenser.
- Add 10 µl Cy5 reaction buffer.
- Add 5 µl of Cy5 dye solution.
- Mix the contents of the tube and incubate overnight in a dark place at room temperature 20 – 30 °C.

4. Purification of Cy5-labeled RNA.

To purify RNA from the components of the reaction with the dye, we perform the second stage of the protocol (point 2 of this protocol).
After drying the precipitate, add the required amount of DEPC-treated water, mix and measure the RNA concentration in any convenient way.

Note: When using a kit for labeling 100 µg of RNA, the output will be a preparation containing 70 - 90 µg of RNA with 50 - 80% labeled RNA.

Storage and transportation conditions

Storage temperature: -20°C. See the expiration date on the packaging.
The kit should be transported at -20°C.