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Ribo488 RNA BR Fluorimetric Assay Kit manual

Contents

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Ribo488 RNA BR Fluorimetric Assay Kit manual

This kit is designed for measuring RNA concentration over a wide range using a fluorimeter (any fluorimeter is suitable; for example, QuReader 1 or QuReader 8).

The kit allows accurate measurement of RNA concentration in the range of 1–1200 ng/µL (RNA quantity for measurement on the fluorimeter: 20–1200 ng of RNA per 200 µL of sample).

The main component of the kit is the fluorescent dye Ribo488, which binds to RNA and exhibits pronounced fluorescence upon complexation. Impurities in small amounts, such as salts, detergents, and solvents, have only a negligible effect on measurement results.

Using the Ribo488 RNA BR Fluorimetric Assay kit is a convenient way to measure RNA over a wide range of concentrations:

- It requires a small volume of the original sample (1–20 µL);
- All measurements are performed at room temperature;
- Depending on the number of samples, measurements take an average of 15–20 minutes;
- The fluorescence of the samples is stable for 3 hours.

The kit includes a 200× concentrate of the fluorescent dye Ribo488, a buffer for preparing the dye's working solution, and RNA Concentration Standards (0 and 100 ng/µL). For convenience, our kit contains thin-walled tubes compatible with a fluorimeter.

To determine protein impurities in the RNA sample, the QuDye® Protein quantification kit can be used.

Kit components

Kit component	Count				
	S2502 40 assays	12502 100 assays	14502 100 assays	52502 500 assays	54502 500 assays
11510, Ribo488 RNA Quantification Reagent, 100 uL	1	—	—	—	—
N2150, TE buffer, 20x, 25 mL	—	—	—	1	—
G2150, TE buffer, 20x, 5 mL	1	1	—	—	—
B0650, RNA quantification standard, 100 ng/uL in TE buffer, 1 mL	1	1	1	3	3
B9650, Quantitative standard, 0 ng/uL in TE buffer, 1 mL	1	1	1	—	—
21510, Ribo488 RNA Quantification Reagent, 250 uL	—	1	1	—	—
S3250, TE buffer, 1x, 50 mL	—	—	1	—	5
51510, Ribo488 RNA Quantification Reagent, 1.25 mL	—	—	—	1	1
G9650, Quantitative standard, 0 ng/uL in TE buffer, 5 mL	—	—	—	1	1

Store at 4 °C. Warm up to RT before use. Transportation: at room temperature for up to 1 week.

Shelf life 12 months.

Before you begin

- For measurement, it is recommended to use an RNA sample purified from double-stranded DNA and single-stranded DNA impurities.
- All measurements using the Ribo488 RNA BR Fluorimetric Assay kit must be performed at room temperature (22–28 °C).
- Before starting work, carefully warm all solutions to room temperature.
- For regular use of the kit, it is recommended to store the Ribo488 RNA Quantification Reagent and 1× TE Buffer at room temperature in a light-protected place, and the RNA Standard solutions at 4 °C.
- Please note that temperature fluctuations of the sample significantly affect the measurement results. Avoid heating the samples; in particular, do not hold the sample tubes in your hands immediately before measurements on the fluorimeter.
- Since even a short stay of the sample tube in the fluorimeter socket contributes to sample heating, perform fluorescence measurements immediately after placing the sample tube in the fluorimeter socket.
- If it is necessary to re-measure the same sample, the tube should be removed from the fluorimeter socket immediately after the measurement and placed back only for measuring the fluorescence intensity.
- Fluorescence intensity measurements should be performed according to the fluorimeter instructions. Depending on the fluorimeter version, the menu items may differ from those listed below.
- The kit allows measurements to be performed on the green channel in fluorimeter mode. QuReader and other fluorimeter models that support excitation at 430–495 nm and emission at 510–580 nm can be used.

Measurement of RNA concentration in the fluorimeter mode

1. Prepare eight 1.5 mL tubes and label them. Prepare a serial dilution panel of the 100 ng/µL RNA Standard by titration according to the following scheme:
   - **100 ng/µL** (100 µL of Standard)
   - **50 ng/µL** (50 µL of 0 ng/µL Standard and 50 µL of 100 ng/µL Standard)
   - **25 ng/µL** (50 µL of 0 ng/µL Standard and 50 µL of 50 ng/µL Standard)
   - **12.5 ng/µL** (50 µL of 0 ng/µL Standard and 50 µL of 25 ng/µL Standard)
   - **6.25 ng/µL** (50 µL of 0 ng/µL Standard and 50 µL of 12.5 ng/µL Standard)
   - **3.12 ng/µL** (50 µL of 0 ng/µL Standard and 50 µL of 6.25 ng/µL Standard)
   - **1.56 ng/µL** (50 µL of 0 ng/µL Standard and 50 µL of 3.12 ng/µL Standard)
   - **0 ng/µL** (100 µL of 0 ng/µL Standard)

   Store the prepared standards for no more than 24 hours at 4 °C.
2. Prepare the dye working solution, calculating that approximately 200 µL will be needed for each experimental sample and for each of the eight RNA Standard solutions. To do this, dilute the 200× Ribo488 dye concentrate 200 times with 1× TE Buffer. For example, to measure 8 RNA Standard solutions and 2 samples, you need to prepare 200 µL × 10 = 2000 µL of working dye solution (mix 10 µL of 200× Ribo488 dye concentrate and 1990 µL of 1× TE Buffer).
3. Prepare eight 0.5 mL tubes for the RNA Standard solutions and one tube for each experimental sample to be measured. Label the tube caps (do not make markings on the walls of the tubes, as this may lead to incorrect determination of fluorescence intensity).
4. Add the working dye solution and the RNA Standard solutions prepared in step 1 to each Standard tube according to the table below:

Volume of working dye solution, µL	Volume of standard RNA solution, µL	Initial concentration of RNA Standard solution	Final RNA concentration in the measured Standard solution

190	10	100 ng/µL	**5 ng/µL**
190	10	50 ng/µL	**2.5 ng/µL**
190	10	25 ng/µL	**1.25 ng/µL**
190	10	12.5 ng/µL	**0.625 ng/µL**
190	10	6.25 ng/µL	**0.3125 ng/µL**
190	10	3.12 ng/µL	**0.156 ng/µL**
190	10	1.56 ng/µL	**0.078 ng/µL**
190	10	0 ng/µL	**0 ng/µL**

13. Add 180–199 µL of the Ribo488 working dye solution and 20–1 µL of the sample to each sample tube, respectively (the final volume in each tube should be 200 µL).
14. Mix all tubes (containing standards and test samples) on a vortex mixer, centrifuge briefly to remove droplets, and incubate for 5 minutes at room temperature.
15. Measure the fluorescence.

Calculation of RNA concentration

1. Construct a calibration curve using the fluorescence level data of the standard solutions in the following coordinates: the x-axis represents the final RNA concentration in the measured standard solution (the final concentrations of the standard solutions are indicated in the last column of the table above); the y-axis represents the fluorescence value.
2. Approximate the data with a linear function and find the parameters A and B of the function. You can use a calculator to calculate RNA concentration for this purpose.
3. The linear equation of the dependence of fluorescence (FL) on concentration (C) is as follows:

   $FL = A \times C + B$;

   where FL is the fluorescence intensity in arbitrary units, C is the RNA concentration, and A and B are the parameters of the linear function.
4. Calculate the RNA concentrations in the experimental sample:

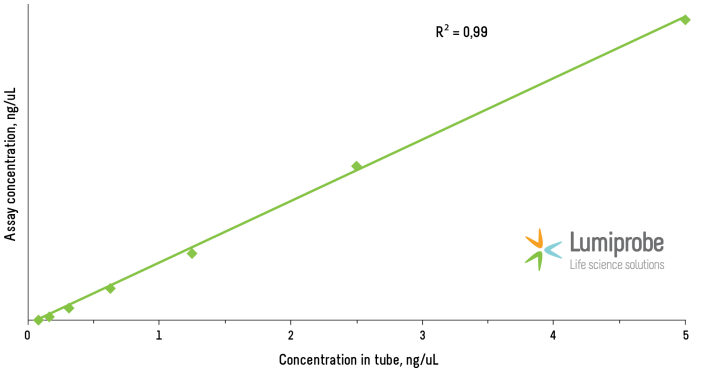
   $C_{sample} = (FL_{sample} - B)/A$;

   where $F_{Lsample}$ is the fluorescence of the sample, and A and B are the parameters of the fitted linear function.
5. Calculate the RNA concentrations in the original sample:

   $C_{ini} = V_{sample} \times C_{sample} / V_{ini}$;

   where V_{sample} is the sample volume, and V_{ini} is the volume of the initial RNA solution used to prepare the experimental sample.

Below are graphs showing the dependence of RFU on the theoretical RNA concentration in the dilution samples and the dependence of the calculated concentration (Assay concentration) on the theoretical RNA concentration in the samples, obtained on the QuReader in fluorescence measurement mode:


R² = 0,99
Assay concentration, ng/uL
Concentration in tube, ng/uL
0
1
2
3
4
5
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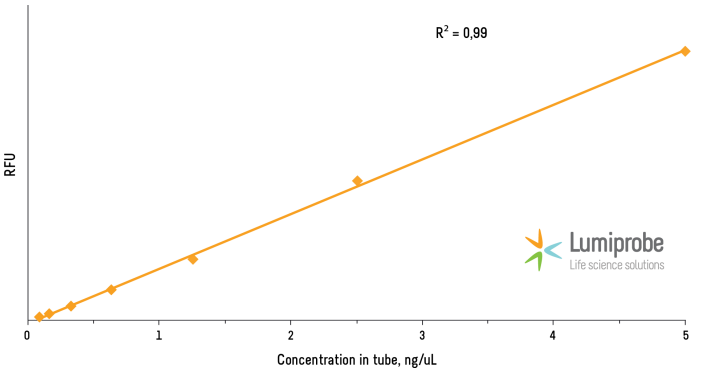
R² = 0,99
RFU
Concentration in tube, ng/uL
0
1
2
3
4
5
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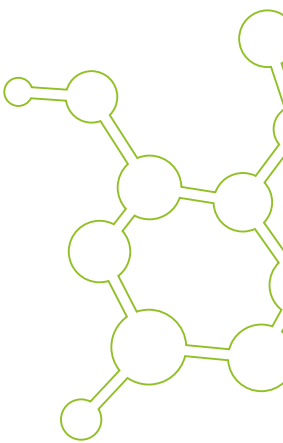

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