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Ribo488 RNA Plate Reader Assay Kit manual

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Ribo488 dye is a highly sensitive and selective reagent that binds to RNA to form a fluorescent complex and allows one to determine the concentration of RNA in solution using a fluorometer. The dye can be used to measure RNA concentration after *in vitro* transcription, before Northern hybridization, in analysis using S1 nuclease, in the preparation of cDNA libraries, before reverse transcription, etc.

The Ribo488 RNA Plate Reader Assay Kit allows to measure RNA concentration over a wide range from 1 ng/mL to 1000 ng/mL. For this purpose, the Ribo488 reagent is used in two dilutions calculated for **high** (20 ng/mL – 1000 ng/mL) and **low** (1 ng/mL – 50 ng/mL) RNA content in samples. The selectivity of Ribo488 binding to RNA allows it to maintain a linear correlation in the presence of many contaminants, such as nucleotides, salts, urea, ethanol, chloroform, detergents, proteins, and agarose. Although Ribo488 also binds to DNA, pretreatment of samples with DNase allows only RNA concentrations to be measured.

This kit is compatible with standard plate and cuvette fluorimeters, as well as NanoDrop™ 3300 and readers versions 3.0 and 4.0.

Kit components

Kit component	Count	
	11520 200 assays	21520 2000 assays
11510, Ribo488 RNA Quantification Reagent, 100 uL	1	—
A0650, RNA quantification standard, 100 ng/uL in TE buffer, 100 uL	1	—
41510, Ribo488 RNA Quantification Reagent, 1 mL	—	1
B0650, RNA quantification standard, 100 ng/uL in TE buffer, 1 mL	—	1
N9150, TE buffer (RNase-free), 20×, 25 mL	1	1

Transportation: at room temperature for 1 week. Store at temperature between 2 °C and 8 °C. Do not freeze!

Shelf life 12 months.

Required materials:

- nuclease-free pipettes and pipette tips (it is recommended to use tips with a filter);
- nuclease-free plastic tubes of 15 and 50 mL, microtubes of 1.5 mL;
- nuclease-free deionized water (for example, treated with diethylpyrocarbonate (DEPC);
- microplates for tablet fluorimeter or cuvettes for cuvette fluorometer.

Protocol

! To eliminate possible dosing errors, we recommend preparing 1× TE buffer and a working solution of the Ribo488 dye with a margin of 10–25 %.

! The level of fluorescence of the RNA/dye complex strongly depends on temperature, so before the experiment, all reagents should be at the same temperature, optimally 22–25 °C.

! This protocol is designed to quantify RNA on a plate fluorimeter but can also be applied to readers (3.0 and 4.0) in fluorimeter mode. If a cuvette fluorimeter is used, the volumes given in this protocol should be increased proportionally depending on the cuvette volume.

1. Preparation of 1× TE buffer

To prepare the required amount of 1x TE buffer, dilute 20x TE buffer with DEPC-treated deionized water. To calculate the required volume of 1× TE buffer, use the table of recommended volumes and the following formula:

$V_{buffer} = V_{sample} \times (N_{samples} + 5) \times 4;$

where V_{buffer} is the required volume of 1× TE buffer, mL; V_{sample} — total sample volume, mL; $N_{samples}$ is the number of experimental RNA solutions, and 5 is the number of standard solutions.

For example, according to the table below, the total sample volume (V_{sample}) for a plate fluorimeter is 0.2 mL. Accordingly, to measure the RNA concentration in 10 experimental solutions, it is necessary to prepare 12 mL of 1× TE buffer.

Recommended volumes for measuring RNA concentration using Ribo488 dye:

Type of equipment		Volume of working solution of Ribo488 dye	Volume of standard RNA solution / test sample	Total sample volume (Vsample)*
Plate fluorimeter	96-well plate*, per well	0.1 mL	0.1 mL	0.2 mL
	24-well plate, per well	0.5 mL	0.5 mL	1 mL
	Other plates	37.5 % of the well volume	37.5 % of the well volume	about 75 % of the well volume
Cuvette fluorimeter	Standard fluorimetric cuvette (3.5 mL)	1 mL	1 mL	2 mL
	Other fluorimetric cuvettes	37.5 % of the cuvette volume	37.5 % of the cuvette volume	about 75 % of the cuvette volume
NanoDrop™ 3300*		0.05 mL	0.05 mL	0.1 mL

**The total sample volume is the sum of the dye solution and the standard/experimental RNA solution mixed in equal volumes.*

2. Preparation of a working solution of the Ribo488 dye

2.1. Thaw and thoroughly mix the contents of the dye tube. Centrifuge off any drippings from the lid.

2.2. Calculate the volume of the working dye solution using the following formula:

$V_{ribo} = ½ \times V_{buffer}$;

where V_{ribo} is the required volume of the working solution of the Ribo488 dye, mL; V_{buffer} is the volume of 1x TE buffer, calculated in step 1 of the current protocol, mL.

For example, to measure 10 experimental RNA solutions on a plate fluorimeter, 6 mL of working dye solution will be required.

2.3. Transfer 1x TE volume equal to V_{ribo} into a new tube and add the dye concentrate from the kit.

- To measure RNA at **high** concentration ranges (20 ng/mL to 1000 ng/mL), dilute the Ribo488 dye concentrate **200**-fold with 1× TE buffer.
- To measure RNA in the **low** concentration range (1 ng/mL — 50 ng/mL), dilute the Ribo488 dye concentrate **2000**-fold with prepared 1× TE buffer.

For example, if 10 experimental solutions are supposed to have a high RNA concentration, 30 µL of Ribo488 concentrate must be added to 6 mL of 1x TE buffer, and if low, then 3 µL of dye concentrate.

! The prepared working dye solution is suitable for use within 3 h.

*! To prepare a working dye solution, use **only** plastic wares. Glassware can sorb the dye on its walls, which will decrease in the dye concentration in the sample and, as a result, lead to distortion of the measurement results.*

3. Preparation of standard RNA solutions

- **To work in the high RNA concentration range** (20 ng/mL to 1000 ng/mL), prepare a 2000 ng/mL RNA stock solution. To do this, add 30 µL of the RNA standard from the kit and 1.47 mL of 1× TE buffer into the tube.

  Next, in the plate, prepare standard RNA solutions from this stock solution at the following concentrations: 1000 ng/mL, 500 ng/mL, 100 ng/mL, 20 ng/mL, and 0 ng/mL. To do this, sequentially add 1x TE buffer and 2000 ng/mL RNA stock solution into the wells of the plate in the amount indicated in the table below.

  Important! *the working dye solution (prepared by diluting the concentrate 200 times in step 2) will be added in step 5 of the protocol.*

  Preparation of RNA standards to construct a calibration line in the range from 20 ng/mL to 1000 ng/mL (**high concentrations range**):

Volume of 1× TE buffer added to the plate well, µL	Volume of RNA stock solution 2000 ng/mL added to the plate well, µL	Volume of the Ribo488 dye working solution added to the plate well, µL	RNA concentration in the standard solution, ng/m
0	100	100	1000
50	50	100	500
90	10	100	100
98	2	100	20
100	0	100	0

- **To work in the low RNA concentration range** (1 ng/mL – 50 ng/mL), prepare a 100 ng/mL RNA stock solution. To do this, add 30 µL of the RNA standard from the kit and 1.47 mL of 1× TE buffer into the tube and mix thoroughly. Next, transfer 75 µL of the prepared RNA solution into a new tube and add 1.425 mL of 1x TE buffer.

  Prepare RNA standard solutions in the plate at the following concentrations: 50 ng/mL, 25 ng/mL, 5 ng/mL, 1 ng/mL, and 0 ng/mL. To do this, sequentially add 1x TE buffer and 100 ng/mL RNA stock solution into the wells of the plate in the amount indicated in the table below.

  Important! *the working dye solution (prepared by diluting the concentrate 2000 times in step 2) will be added later in step 5 of this protocol.*

  Preparation of RNA standards to construct a calibration line in the range from 1 ng/mL – 50 ng/ml (**low concentrations range**):

Volume of 1× TE buffer added to the plate well, µL	Volume of RNA stock solution 00 ng/m added to the plate well, µL	Volume of the Ribo488 dye working solution added to the plate well, µL	RNA concentration in the standard solution, ng/mL
0	100	100	50
50	50	100	25
90	10	100	5
98	2	100	1
100	0	100	0

4. Preparation of experimental samples

4.1. Dilute the RNA samples in 1× TE buffer so that the final volume for each sample is 100 µL, and transfer them to the wells of the plate.

4.2. Add 100 µL of Ribo488 dye working solution to standard RNA solutions and experimental samples. Mix all solutions by pipetting.

4.3. Incubate the prepared RNA standards and experimental samples for 5 min at room temperature.

5. Measurement of fluorescence intensity of standard RNA solutions and experimental samples

Since the Ribo488 dye complex with RNA has an absorption maximum at a wavelength of 493 nm and an emission maximum at 525 nm, the recommended excitation wavelength for setup is 480 nm, and the emission wavelength is 520 nm.

The fluorimeter sensitivity should be adjusted to the standard with the highest RNA concentration (50 ng/mL or 1000 ng/mL) to ensure that fluorescence readings remain within the detection range. The fluorescence level should be close to the maximum RFU of the device. The fluorimeter gain settings should be increased for optimal sensitivity when working with RNA in the **low** concentration range.

6. Calculation of RNA concentration

6.1. Subtract the fluorescence value for the standards without RNA from the fluorescence value for the samples containing RNA. Use this data to make a calibration curve.

6.2. Plot a calibration curve using data on the fluorescence level of standard solutions in coordinates: along the abscissa axis (x) — the final concentration of RNA in the standard solution; along the ordinate (y) — the fluorescence value.

6.3. Approximate the data with a linear function and find the parameters of the functions A and B. You can use our calculator to calculate the RNA concentration.

6.4. The linear equation for fluorescence (FL) versus 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.

6.5. Determination of RNA concentration in experimental sample:

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

where FL_{sample} is the sample's fluorescence, and A and B are the parameters of the found linear function.

6.6. Determination of RNA concentration in the initial sample:

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

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

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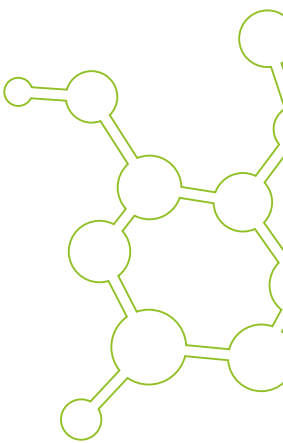

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