

RNA Quantification High Sensitivity Kit (Half-Volume 100 μ L Assay)

(For use with the Qubit Fluorometer)

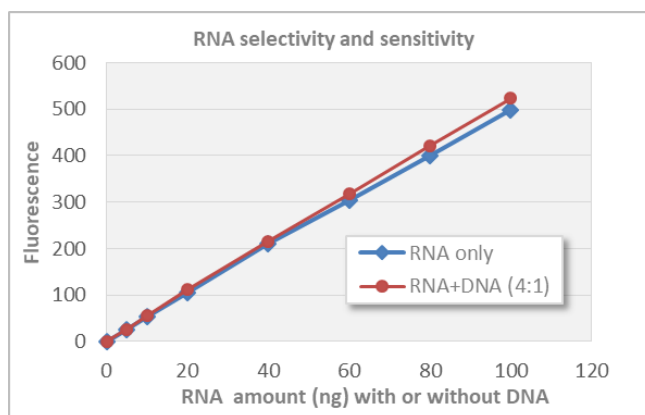
Catalog No. 40214S: 200 assays

Catalog No. 40214L: 1000 assays

Component storage: store at 4°C and room temperature upon receiving

Description

The **RNA Quantification High Sensitivity Kit (Half-Volume 100 μ L Assay)** is developed for RNA quantification. The RNA Quantification HS kit includes HS Dye, HS Dilution Buffer, and two RNA Standards. Simply dilute the HS Dye with the HS Dilution Buffer, add RNA sample, then read the concentration using the Qubit Fluorometer. The assay is accurate for RNA concentrations from 250 pg/ μ L to 100 ng/ μ L.



Feature

- Reduced assay volume to 100 μ L
- Cut the costs to 50% as compared to standard 200- μ L assays
- High-throughput compatible: The 96-well plate racks and multichannel pipettes can be used.
- Shorten assay preparation time by 8-12 folds.

Common contaminants such as salts, free nucleotides, solvents, detergents, or protein are well tolerated in the assay (Table 1).

Table 1. Effect of contaminants in the RNA HS Assay

Contaminant	Final concentration in the assay	Concentration in 10- μ L sample	Result
Sodium chloride	10 mM	200 mM	OK
Magnesium chloride	40 μ M	0.8 mM	OK
Sodium acetate	10 mM	200 mM	OK
Ethanol	1%	20%	OK
Phenol	0.10%	2%	OK
SDS	0.005%	0.10%	OK
Triton X-100	0.0010%	0.02%	OK
dNTP	100 μ M	2 mM	OK
BSA	20 μ g/mL	400 μ g/mL	OK
DNA	0.25X	0.25X	OK

Component

	Cat.# 40214S	Cat.# 40214L
• RNA HS Dilution Buffer	50 mL	250 mL
• RNA HS Dye	0.25 mL	1.25 mL
• RNA HS Standard 1 (0 ng/μL)	1 mL	5 mL
• RNA HS Standard 2 (10 ng/μL)	1 mL	5 mL

Storage Condition

- RNA HS Dye (protect from light) and RNA HS Standards: store at 4°C, stable for 6 months.
- RNA HS Dilution Buffer: store at room temperature, stable for 6 months.

Reagent & Equipment Needed (not provided in this kit)

- Plastic container (disposable) for diluting the HS Dye with the HS Dilution Buffer
- 96-well PCR tube racks and lids
- Assay Tube Adaptor (Half-Volume 100 μL Assay): BioDynami Cat. # 40357
- Assay Tubes (Half-Volume 100 μL Assay): BioDynami Cat. # 40333S

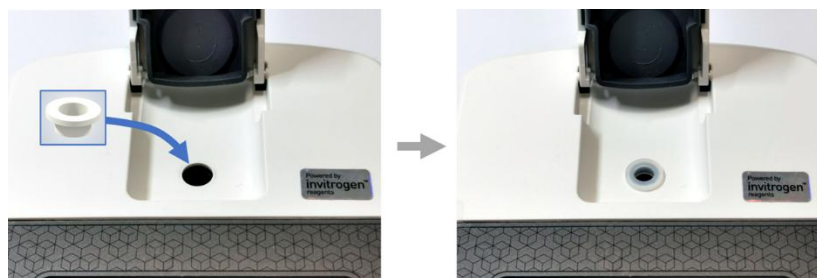
Assay Parameters

- **Assay temperature:** The assays are designed to be performed at room temperature (22-28°C). Store the Dilution Buffer at room temperature.
- **Incubation time:** To get optimal fluorescence, incubate the assay tubes for 2 minutes after mixing the sample or standard with the working solution. The fluorescence signal is stable for 1 hour at room temperature after the incubation period.
- **Reagent photobleaching:** The reagent has high photostability with <0.3% fluorescence drop after 5 readings. However, if the assay tube remains in the fluorometer for multiple readings, the fluorescence will decrease as the temperature of solution will increase. If multiple readings of a single tube are needed, remove the tube from the fluorometer and wait for 30 seconds before taking next reading.

Protocol

Note:

- Assay Tube Adaptor (BioDynamy Cat. # 40357) and Assay Tubes (for Half-Volume 100 μ L Assay, BioDynamy Cat. # 40333S) required. Sold separately.
 - **1X working solution** can be stored at 4°C and protected from light, stable for up to 1 month.
- 1) Label the tube lids for two standards and samples. Do not label the side of the tubes as this could interfere with the sample read.
 - 2) Prepare the **1X working solution** by diluting the RNA HS Dye 1:200 in RNA HS Dilution Buffer in a clean plastic tube (do not use glass container). Each standard requires 95 μ L and each sample tube requires 90-99 μ L of working solution.
 - 3) Place the assay tubes on 96-well PCR tube racks.
 - 4) Standard and sample preparation:
 - Standard tube preparation: Add 95 μ L of working solution to the tubes for standards. Vortex BR Standards for 2–3 seconds, add 5 μ L of each standard to the appropriate tube, and close the tube caps.
 - Sample tube preparation: Sample volume can be 0.5-10 μ L. Please note that smaller sample volume (<1 μ L) may generate more variations. Add the working solution to the assay tubes so that the final volume in each tube after adding the sample is 100 μ L. Add the sample to the assay tube and close the tube caps. The final volume in each tube should be 100 μ L.
 - 5) Cover the 96-well PCR tube racks with the rack lid, mix by vortexing the racks for 10 seconds. Please make sure no air bubbles in the solution after vortexing.
 - 6) Incubate tubes at room temperature for 2 minutes.
 - 7) Place the Assay Tube Adaptor in the sample chamber of the fluorometer as shown below:



- 8) Read standards: follow the procedure for Qubit Fluorometer.
 - 9) Read samples: follow the procedure for Qubit Fluorometer.
- Attention: Selection of sample volume:** The samples are diluted in 1X working solution, and selection of sample volume is needed. The fluorometer RNA HS setting is for 200 μ L of reagent, not for 100 μ L of reagent. The sample volume diluted in 100 μ L is equivalent to the double sample volume diluted in 200 μ L. For example, 1 μ L of sample diluted in 100 μ L is equivalent to 2 μ L of sample diluted in 200 μ L.

Selection of sample volume: choose 2X of the actual sample volume.

For example: if your actual sample volume is 1 μ L, choose 2 μ L as sample volume; if your actual sample volume is 5 μ L, choose 10 μ L as sample volume.

Quality Control

Kit components passed stringent functional quality test.

Product Use Limitation

This product is developed and sold for research purposes and *in vitro* use only. Please refer to BioDynami.com for Material Safety Data Sheet of the product.

Limited Label License

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Note: Qubit is a registered trademark of Thermo Fisher Scientific.

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