DeNovix RNA Assay Instructions

Technical Note 199

Introduction

The DeNovix RNA Assay enables accurate detection of RNA samples with a standard detection range from 5 to 1000 ng total mass in 200 µL volumes. This equates to sample concentrations of 0.5 ng/µL to 100 ng/µL when using 10 µL sample volumes in a 200 µL total assay volume.

The upper detection limit can be extended to 1500 ng/µL by adding 1 µL of a 1500 ng/µL sample to 199 µL of working reagent. The lower limit can be extended to 0.25 ng/µL by adding 20 µL of 0.25 ng/µL sample to 180 µL of the working reagent.

Kit Contents

Kits are available in 1000, 250, and 50 (evaluation size) assays and include these components:

Component	1000	200	EVAL
DeNovix RNA Assay Quantitation Dye (200x)	1 mL	250 µL	50 µL
DeNovix RNA Assay Buffer	250 mL	62.5 mL	12.5 mL
100 ng/µL RNA Standard (mammalian cell)	4 x 400 µL	1 x 400 µL	0.1 mL
RNA Standard, 0 ng/µL	2 mL	0.5 mL	0.5 mL

Best Practices

- Use calibrated pipettes and RNase-free pipette tips.
- Prepare the working solution fresh for each assay.
- Ensure all samples and standards are treated identically in terms of incubation times and temperature.
- Avoid introducing air bubbles when mixing.
- Generate a new standard curve for each assay.
- Assay total mass must be considered when deciding how much sample to use. This assay is appropriate for 0.5 - 1500 ng total mass per tube.
- Label the top, not the sides of the assay tubes.

See Technical Note 198 for additional information regarding solvent compatibility and multi-point curves.

Sample Prep

1. Equilibrate all solutions to room temperature before use. Vortex, then centrifuge vials briefly to minimize reagent loss on the cap.
2. Prepare working solution by mixing 10 mL of the assay buffer with 50 µL of the dye. Scale volumes as needed to make enough volume to aliquot 190 µL of the mixture for each standard and unknown.
3. For each standard or unknown sample, add 190 µL of the working solution to a labeled tube. Adjust volume when adding more or less than 10 µL of the unknown sample.
4. Use thin-walled, clear UV-transparent 0.5 mL PCR tubes for assay measurements (DeNovix cat# TUBE-PCR-0.5-500 or equivalent).
5. Add 10 µL of the 0 ng/µL and 100 ng/µL standards and 1-20 µL of unknown RNA samples to the respective tubes and mix well.
6. Incubate assay tubes at room temperature for 5 minutes.

Sample Measurement

1. Launch the Fluoro RNA app using a DeNovix fluorometer.
2. Use the drop-down menu to select the **DeNovix RNA Assay**.
3. Select **Preconfigured 2 Standards** and then choose **Generate New Standard Curve**.
4. Insert the 0 ng/µL RNA standard, lower the lid and tap **Measure**.
5. Insert the 100 ng/µL RNA standard, lower the lid and tap **Measure**.
6. After both standards are measured, tap the **Samples** button, insert a sample tube and tap **Measure**.

Reagent Storage

Component	Protect from Light	Temperature
DeNovix RNA Assay Quantitation Dye (200x)*	Yes	4°C - Room Temperature
DeNovix RNA Assay Buffer	Optional	4°C - Room Temperature
100 ng/µL RNA Standards	Yes	-20°C
0 ng/µL Standards	Yes	4°C - Room Temperature

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Version: Nov 2019