Technical Note 199

DeNovix RNA Assay Short Protocol

Introduction

The DeNovix RNA Assay enables accurate detection of purified RNA samples with a detection range from 5 ng to 1500 ng total mass in 200 µL volumes. This equates to sample concentrations of 0.25 ng/µL to 1500 ng/µL when using 1 – 20 µL sample volumes in a 200 µL total assay volumes.

The assay is linear for sample concentrations as high as 1500 ng/µL when adjusting volumes to 1 µL of sample into 199 µL of working reagent. Total mass should not exceed 1500 ng for best results.

Quantification of RNA sample concentrations as low as 0.25 ng/µL can be achieved by adding 20 µL of the 0.25 ng/µL sample to 180 µL of the working reagent.

These results are applicable to the DeNovix QFX Fluorometer, DS-11 FX, DS-11 FX+, and FX module.

Kit Contents

Three assay sizes are available. The volume of components in each kit are sufficient for 1000, 250 or 50 (evaluation size) assays respectively. Kit components are shown in the table below.

Table 1. Kit sizes and the amount of each component contained within each kit.

Component	1000	250	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
RNA Standard, 100 ng/µL	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

It is important to pay careful attention to pipetting accuracy and overall sample handling techniques when preparing samples for the RNA Fluorescence Quantification Assay.

- Prepare the working solution fresh for each assay. Discard the solution after 12 hours.
- Use properly calibrated pipettes and RNase-free pipette tips for best accuracy.
- Use thin-walled, clear UV compatible 0.5 mL PCR tubes (DeNovix Cat. No # TUBE-PCR-0.5-500 or equivalent) or black-walled 96 well microplates.
- Do not label the side of an assay tube as this could interfere with the sample measurement.
- Avoid introducing air bubbles into the sample solution when mixing samples.
- Minimize assay tube and solution temperature fluctuations.
- Ensure all samples and standards are treated identically in terms of incubation times and temperature.
- Ensure all sample concentrations in the assay tubes or microplate wells fall within the limits of the reagent kit.

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

The kit is stable for at least 6 months from ship date when stored as recommended.

Table 3. Storage conditions for each component in the DeNovix RNA assay kit.

Component	Protect from Light	Temperature
DeNovix RNA Assay Dye (200x)*	Yes	4°C - Room Temperature
DeNovix RNA Assay Buffer	Optional	4°C - Room Temperature
RNA Standard, 0 ng/µL	No	4°C - Room Temperature
RNA Standard, 100 ng/µL	Yes	-20°C

*Note: The DeNovix RNA Assay Dye is provided in DMSO, which may freeze if stored at 4°C.

DeNovix dsDNA Quantification Assays

In addition the DeNovix RNA Assay, DeNovix also offers a range of dsDNA fluorescence quantification assays. The range of assays offered is indicated in Table 6.

Assay Detection Ranges

DeNovix dsDNA Assay	Range
Broad Range	0.1 – 2000 ng/µL
High Sensitivity	10 pg/µL – 250 ng/µL
Ultra High Sensitivity	0.5 – 300 pg/µL

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