DeNovix RNA Assay
Technical Note 198

Introduction

The DeNovix RNA Assay enables accurate detection of purified 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 1000 ng/µL when using 10 µL sample volumes in a 200 µL total assay volume.

Extended Range

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 (1500 ng total mass) and the lower detection limit can be extended to 0.25 ng/µL using 20 µL in 180 µL (0.5 ng total mass).

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 (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

Instrument Compatibility

The spectral properties of the dye are excitation/emission of 634/671 nm in the presence of RNA.

The kit is compatible with fluorescence microplate readers and fluorometers with the appropriate excitation sources and emission detectors.

Specific instructions using the 2 point standard assay with DeNovix DS-11 FX , FX module or the QFX fluorometer are included in Technical Note 199.

Assay Considerations

Mammalian cell RNA is provided as the reference standard. It may be preferable to use an alternative RNA standard more similar (i.e similar size, linear vs. non-linear i.e. tRNA and rRNA) to the unknown samples of interest. For bacterial RNA, consider using a species-specific standard as the strand construction varies widely depending on the species.

Although many instruments including DeNovix DS-11 fluorometers offer the option to use previously saved values, it is recommended that a new standard curve be generated at the time of the assay for optimal results.

Assay Linearity and Detection Limits

Fluorescent quantification specifications are often expressed in a variety of conventions. The full detection range (including the extended range) of this assay can be expressed in the following specifications:

Table 2. Tolerance of the assay in total mass added to each tube and the corresponding concentration from the sample.

Specification	Range
Absolute mass per assay tube	0.5 ng to 1500 ng per 200 µL
Concentration in sample stock tube	250 pg/µL to 1500 ng/µL

Reagent Storage

The kit is stable for at least 6 months from ship date when stored as recommended.
*Note: The DeNovix RNA Assay Dye is provided in DMSO, which may freeze if stored at 4°C.

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

Best Practices

- 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.

Assay Protocol

1. Allow all solutions to equilibrate to room temperature before use.
2. Vortex, then centrifuge vials briefly before opening to minimize reagent loss on the cap.
3. Prepare working solution by mixing 200 µL of the assay buffer with 1 µL of the dye, scaling for the number of samples being analyzed.
4. Scale volumes as needed to make enough volume to aliquot 190 µL of the mixture per standard and unknown to be measured.
5. For each standard or unknown sample, add 190 µL of the working solution to a labeled tube or micro well. Adjust volume when adding more or less than 10 µL of the unknown sample.
6. Add 10 µL of the 0 ng/µL and 100 ng/µL standards and 1-20 µL of unknown DNA samples to the respective tubes and mix well.
7. Incubate standards and samples at room temperature for 10 minutes, while protecting them from light.
8. Generate the standard curve and then measure the samples using the proper excitation source and emission filters.

Standard Dilutions

Preparing diluted standards is not required when using the optimized preconfigured 2 point assay option in the DeNovix FX or QFX software. For the DeNovix User Defined Standards option or for use on microplate readers, prepare RNA standards by serial dilution of the 100 ng/µL standard provided in DEPC water.

Table 4. Constructing a multi-point standard curve from the RNA standard provided.

Standard	RNA	DEPC Water
100 ng/µL	Undiluted stock tube	None
50 ng/µL	20 µL of 100 ng/µL standard	20 µL
25 ng/µL	10 µL of 100 ng/µL standard	30 µL
10 ng/µL	5 µL of 100 ng/µL standard	45 µL
2 ng/µL	10 µL of 10 ng/µL standard	40 µL
0.5 ng/µL	10 µL of 2 ng/µL standard	30 µL
0 ng/µL	None	100 µL

Data Analysis

Sample concentrations are automatically calculated when using a DeNovix DS-11 FX or QFX fluorometer.

For all other instruments, follow the instructions below:

1. Generate a standard curve to determine the unknown RNA concentration.
2. Average replicates values for each sample and subtract the average zero RNA value from each data point.
3. Plot the fluorescence RFU values for the RNA standards on the y-axis and ng/well RNA on the x-axis, and fit a trend line (Figure 2) through these points to generate a standard curve with a y-intercept = 0.
4. Use the equation for the trend line to calculate the amount of unknown RNA in each well (y = fluorescence and x = ng RNA per well or tube).

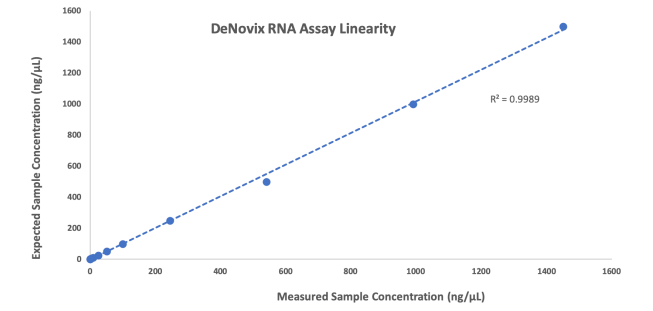

Fig. 2. *E. coli* total RNA measured using the DeNovix RNA Assay on a DS-11 FX fluorometer.

Solvent Compatibility

Table 5. The tolerance of the dye in the presence of common solvents

Compound	Final maximum concentration in assay (200 µL)
Ammonium Acetate	5 mM
Sodium Chloride	50 mM
Ethanol	0.5%
Phenol	0.1%
SDS	0.01%
dNTPs*	100 µM

Troubleshooting

- Review the Best Practices recommendations.
- Confirm tubes or assay plates are UV transparent,
- Confirm that the correct excitation source and emission filters were used at the time of the measurement.
  - Note: The DeNovix DS-11 and QFX software automatically uses the correct LED and emission filter.
- Confirm that standard concentrations and dilutions are performed correctly.
- Confirm that the correct concentration units for the standard curve and the unknown samples are used to calculate the stock concentrations.
- If applicable, ensure that the correct dilution factor or sample volume added value is entered into the appropriate Run screen field before a measurement is made.

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 X.

Table 6. Other fluorescent assays offered by DeNovix to detect dsDNA.

DeNovix dsDNA Assay	Range
Broad Range	100 pg/µL to 2000 ng/µL
High Sensitivity	10 pg/µL to 250 ng/µL
Ultra High Sensitivity	0.5 pg/µL to 300 pg/µL

Customer Support

Contact DeNovix Customer Support if further help is required. Outside of the US, please contact your local distributor for assistance.

Instructions specific to performing a two-point standard curve assay on a DeNovix fluorometer (Technical note 199) is available at www.denovix.com.

DeNovix Inc.
3411 Silverside Road
Wilmington, DE 19810 USA

Phone:+1.302.442.6911
Email:info@denovix.com
www.denovix.com

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