
**Technical Note 198**

# DeNovix RNA Assay 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 volume.

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. Safety data sheets are available at denovix.com/sds.

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 |

## 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 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 Series 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 |
|---|---|

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|---|---|
| Absolute mass per assay tube | 5 ng to 1500 ng per 200 µL |
| Concentration in sample stock tube | 0.25 ng/µL to 1500 ng/µL |

## Reagent Storage

The assay is stable for 12 months from ship date. The RNA standard is recommended to be stored at -80°C, however can be stored at -20°C with a shelf life reduced to 6 months.

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 | -80°C |

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

## 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.

## 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 the dye with the assay buffer in a 1:200 ratio, e.g. 50 uL dye into 10 mL buffer.
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 RNA samples to the respective tubes and mix well.
7. Incubate assay tubes at room temperature for 5 minutes. Protect from light.
8. Generate the standard curve and then measure the samples using the proper excitation source and emission filters.

## Recommended Sample Volume

These recommendations ensure that sample concentrations are within the total mass detection limits of the assay.

| Initial Sample Concentration | Recommended Sample Volume |
|---|---|
| 0.5 – 150 ng/µL | 10 µL |
| 0.25 – 5 ng/µL | 20 µL |
| 100 – 750 ng/µL | 2 µL |
| 750 – 1500 ng/µL | 1 µL |

## 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 |

| Standard | RNA | DEPC Water |
|---|---|---|
| 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 1) 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).

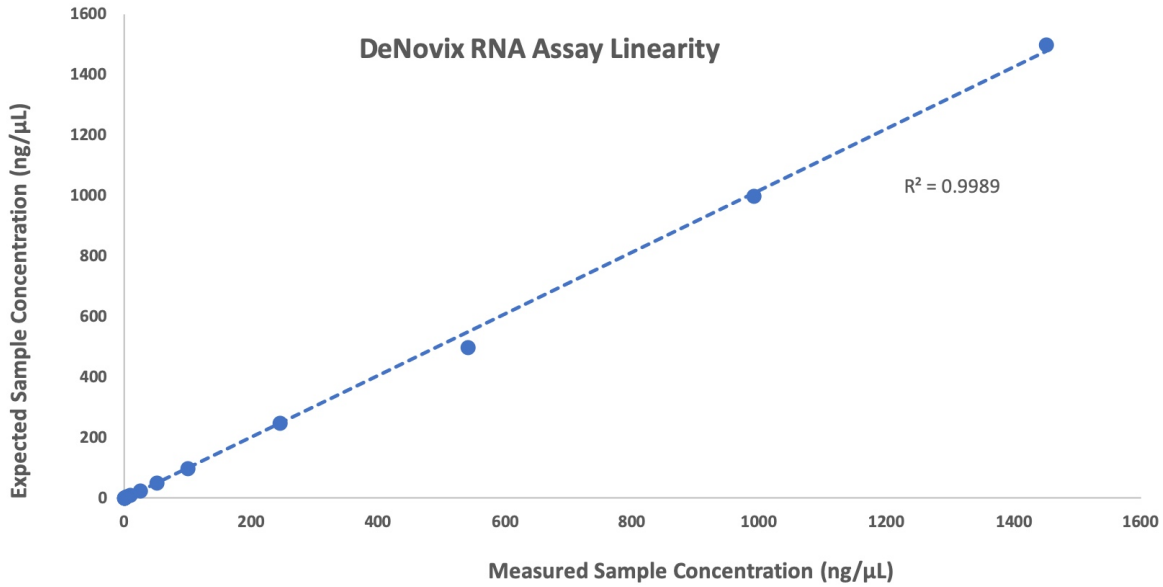


Figure 1. E. coli total RNA measured using the DeNovix RNA Assay on a DS-11FX fluorometer.

## Solvent Compatibility

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

| Compound | Final Concentration in Assay (200 µL) |
|---|---|
| Sodium Chloride | 10 mM |
| Magnesium Chloride | 2 mM* |
| Sodium Acetate | 2 mM |
| Ammonium Acetate | 10 mM |
| Ethanol | 0.10% |
| SDS | 0.01%* |
| Triton X-100 | 0.001%* |
| CTAB | 0.0005%* |
| BSA | 20 ug/mL |

* This contaminant caused more than 10% change in fluorescence when added to a sample at the given concentration.

## 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 Fluorometer 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 6.

Table 6: DeNovix dsDNA Assays

Assay Detection Ranges

| DeNovix dsDNA Assay | Range |
|---|---|
| Broad Range | 0.1 – 2000 ng/µL (extended range to 4000 ng/uL) |
| High Sensitivity | 10 pg/µL – 250 ng/µL (extended range down to 5 pg/uL) |
| Ultra High Sensitivity | 0.5 – 300 pg/µL |

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