Protocol

NanoDrop Ultra RNA BR Fluorescence Assay

Introduction

The Thermo Scientific™ NanoDrop™ Ultra RNA BR (Broad Range) Fluorescence Assay, when used with either the Thermo Scientific™ NanoDrop™ Ultra FL or the Thermo Scientific™ NanoDrop™ Ultra^{C} FL Spectrophotometer and Fluorometer (For use with NanoDrop Ultra software version 1.2.0 and above), helps provide an accurate and selective method for the quantitation of total RNA, rRNA, or large mRNA. This protocol contains two options for use with this assay kit, which together provide accurate results for initial RNA sample concentrations ranging from 10 to 1,000 ng/µL.

Contents and storage

Component	Total volume (250 reactions)	Concentration	Storage[1]
NanoDrop Ultra RNA BR Fluorescence reagent (component A)	70 µL	200X in DMSO	Room temperature[2] desiccate Protect from light
NanoDrop Ultra RNA BR Fluorescence buffer (component B)	15 mL	Not applicable	≤30°C
NanoDrop Ultra RNA BR Fluorescence standard #1 (component C)	500 µL	0 ng/µL in TE buffer	2°C to 8°C[3] Avoid freeze/thaw cycles
NanoDrop Ultra RNA BR Fluorescence standard #2 (component D)	500 µL	100 ng/µL in TE buffer	

[1] When stored as directed, kits are stable for 6 months.
[2] For long-term storage, the NanoDrop Ultra RNA BR reagent can be stored at ≤–20°C
[3] For long-term storage, store the rRNA standards at ≤–20°C or –70°C.

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Critical assay parameters

- **Assay temperature**
  The NanoDrop Ultra RNA BR Fluorescence Assay helps deliver optimal performance when all solutions are at room temperature; temperature fluctuations can influence the accuracy of the assay. To minimize temperature fluctuations, do not hold the assay tube in your hand before reading because this warms the solution and will result in a different reading.
- **Incubation time**
  To allow the NanoDrop Ultra RNA BR Fluorescence Assay to reach optimal fluorescence, incubate the tubes for 5 minutes after mixing the sample or standard with the working solution.
- **Curve-fitting algorithm**
  A curve-fitting algorithm which requires two standards is used in the calculation of concentration data. The NanoDrop Ultra RNA BR Fluorescence Assay uses a modified Hill plot to generate concentration data based on the relationship between the two standards used.
- **Assay selectivity**
  The NanoDrop Ultra RNA BR Fluorescence Assay is highly selective for RNA over double-stranded DNA (dsDNA).
- **Effects of contaminating substances**
  When working with untested contaminating substances, and to achieve highest accuracy, the standards should be assayed under the same conditions as the experimental samples. For example, if the experimental samples are in an untested buffer and 2 µL of each sample is used, add 2 µL of the untested buffer (lacking RNA) to each standard.

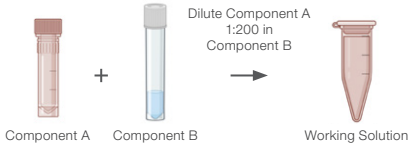
Supplies needed

- **Equipment**
  - NanoDrop Ultra FL or NanoDrop Ultra^{C} FL Spectrophotometer and Fluorometer
  - RNAse-free, calibrated pipettes. It is recommended to chose a pipette that will dispense liquid in the upper part of it's range. For example, use a P20 to dispense 18 µL instead of a P100.
- **Materials**
  - RNAse-free low retention pipette tips
  - Lint-free laboratory wipes
  - 0.5 mL microcentrifuge tubes and caps (amber recommended)
- **Reagents**
  - NanoDrop Ultra RNA BR Fluorescence Assay (NDU-FL-RNA-BR)
  - 1X TE buffer

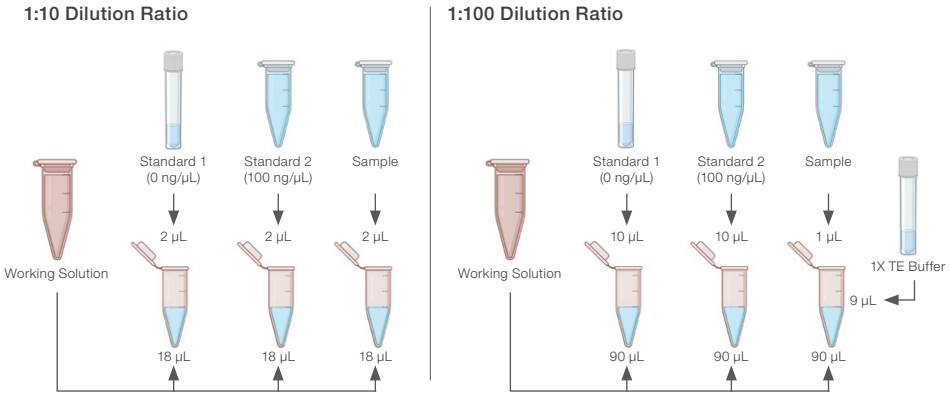
Sample and standard preparation

Component Preparation

1) Prepare a working solution



Standard and Sample Preparation



1. Set up the required number of tubes for any standards and samples that will be used. The NanoDrop Ultra RNA BR Fluorescence Assay requires 2 standards.
2. Ensure that each tube is labeled appropriately.
3. Prepare a working solution by diluting **Component A** 1:200 in **Component B**. Use a clean plastic tube each time you prepare a working solution of these reagents.

Note: The Reagent Calculator found within the Fluorescence tab can be used to quickly calculate the volumes needed of each component to ensure enough working solution is made.

Note: Do not mix the working solution in a glass container.

4. Using the chart below, add the appropriate amounts of working solution, 1X TE buffer, standard, and sample to each of the prepared tubes such that the final volume is 20 µL (1:10 sample to reaction mix) or 100 µL (1:100 sample to reaction mix) in each tube.

Note: If you are unsure which option to use, please first measure the absorbance of your samples using the applicable nucleic acid absorbance application. Use the calculated sample concentration to identify in which range your sample falls.

Sample and standard preparation (continued)

	1:10 (Sample concentrations between 10–100 ng/µL)		1:100 (Sample concentrations between 100–1,000 ng/µL)	
	Standard assay tubes	Sample assay tubes	Standard assay tubes	Sample assay tubes
Volume of working solution	18 µL	18 µL	90 µL	90 µL
Volume of 1X TE buffer	–	–	–	9 µL
Volume of standard	2 µL	–	10 µL	–
Volume of sample	–	2 µL	–	1 µL
Total volume in each assay tube	20 µL	20 µL	100 µL	100 µL

Note: The final volume in each tube must be either 20 µL (1:10 sample to reaction mix) or 100 µL (1:100 sample to reaction mix). Prepare sufficient working solution to accommodate all standards and samples.

For example, when using the 1:10 option for 8 samples, prepare enough working solution for the samples plus 2 standards and include some overage: ~ 18 µL per tube in 10 tubes yields 180 µL of working solution (0.9 µL of **Component A** in 179.1 µL of **Component B**). However, as it not recommended to pipette volumes less than 1 µL, in this case the user should use 1 µL of **Component A** in 199 µL of **Component B** to create the working solution.

The NanoDrop Ultra software includes a reagent calculator, which quickly computes the necessary volume of working solution needed. Ensure you select the correct assay and total assay volume options.

9 µL of TE is used in the 1:100 sample to reaction mix option to make sure the dye concentration is consistent between the dilution schemes.

5. Vortex each tube for < 3 seconds with care taken not to create bubbles. Centrifuge briefly.
6. Allow all tubes to incubate at room temperature for 5 minutes, then proceed to **RNA: Broad range procedure** (next section).

RNA: Broad range procedure

1. Lift the instrument arm and clean the upper and lower pedestal with a new laboratory wipe.
2. Lower the arm.
3. From the home screen, select the **Fluorescence tab**, followed by **RNA Fluorescence**.
4. Configure the measurement parameters.
    - Enable or disable Auto Naming using the provided toggle.
    - Indicate the desired quantity of replicate measurements for the standards by using the + and - buttons located next to the Replicates option.

Note: For best performance, it is recommended to measure 5 replicates of standards and samples.

    - Select the desired Sample to Reaction Volume option by selecting from the drop-down menu and then select **Save**.

Note: After the standards are measured the Sample to Reaction Volume option can be changed for each sample by selecting ⚙ at the top of the screen if desired.

    - Proceed to step 5.

5. Pipette 2 µL of the prepared tube containing Standard #1 onto the lower pedestal, lower arm.

Note: If the **Auto-Measure** feature is OFF, select **Measure** to begin the measurement. If the **Auto-Measure** feature is ON, the measurement will begin automatically after the arm is lowered.

6. After the measurement is complete, remove the sample from both upper and lower pedestal using a dry laboratory wipe.
7. Repeat steps 5 & 6 to measure any replicates for **Standard #1** (following the on-screen prompts
    – Always use a fresh 2 µL aliquot for each measurement.
8. Pipette 2 µL of the prepared tube containing **Standard #2** onto the lower pedestal, lower arm.

Note: If the **Auto-Measure** feature is OFF, select **Measure** to begin the measurement. If the **Auto-Measure** feature is ON, the measurement will begin automatically after the arm is lowered.

9. After the measurement is complete, remove the sample from both upper and lower pedestal using a dry laboratory wipe.
10. Repeat steps 8 & 9 to measure any replicates for **Standard #2** (following the on-screen prompts).
    – Always use a fresh 2 µL aliquot for each measurement.

RNA: Broad range procedure (continued)

11. When all standard measurements have been completed, the Standards Completed window will appear with 2 options.

    –Select **Remeasure Standards** to rerun any previous standard measurements deemed unacceptable.

    - From the standards measurement screen, standard curve screen, or standards data table, press and hold the row to show the **Sample Details** window.
    - Select **Remeasure** then select **Yes** to confirm.

    Note: Remeasuring a standard will overwrite the previously recorded data for that standard.

    Only one standard can be remeasured at a time.

    - Repeat this process for all applicable standards.

    – After all standards have been measured, select **Measure Samples** and proceed to step 12.

12. Enter a sample ID at the top of the screen and pipette 2 µL of the corresponding sample tube onto the lower pedestal, lower arm.

    Note: If the **Auto-Measure** feature is OFF, select **Measure** to begin the measurement. If the **Auto-Measure** feature is ON, the measurement will begin automatically after the arm is lowered.

    It is not necessary to blank the instrument between measurement of the standards and the unknown samples.

    When the sample measurement is completed, the reported values are displayed.

13. After the measurement is complete, remove sample from both upper and lower pedestal using a dry laboratory wipe.
14. Repeat steps 12 & 13 to measure all remaining replicates and samples (following the on-screen prompts).

    – Always use a fresh 2 µL aliquot for each measurement.

15. When finished, select **End Experiment**.
16. The experiment name can be changed at this time and up to five unique identifiers/tags can be added; once complete select **Next**.
17. Results can be exported and printed at this time by selecting **Continue**, or at a later time from the History.

    – After exporting or printing, select **Done** to go back to the home screen.

18. If results do not need to be exported or printed, select **Finish** then **Done** to return to the home screen.

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