Technical Note 142

Broad Range Assay Standard Protocol

Introduction

The DeNovix dsDNA Broad Range Assay enables the accurate detection of double-stranded DNA (dsDNA) samples with a standard detection range from 2 to 2000 ng total mass in 200 µL volumes. This equates to sample concentrations of 0.1 – 2000 ng/µL when using 1 – 20 µL sample volumes in a 200 µL total assay volume.

The upper detection limit can be extended to 4000 ng/µL by adding 1 µL of a 4000 ng/µL sample to 199 µL of working reagent. There is some loss of linearity with this assay when adding more than 2000 ng total mass per assay tube.

View Detailed Protocol

Kit Contents

Kits are available in 1000, 250 and 50 (evaluation size) assays and include the components in Table 1. Safety data sheets are available at denovix.com/sds.

Component	1000	250	EVAL
DeNovix dsDNA Broad Range Dye (100x)	2 x 1 mL	0.5 mL	100 µL
DeNovix dsDNA Broad Range Buffer	250 mL	50 mL	10 mL
DeNovix dsDNA Broad Range Enhancer (100x)	2 x 1 mL	0.5 mL	100 µL
200 ng/µL dsDNA Standard (calf thymus)	2 mL	1 mL	0.5 mL
0 ng/µL dsDNA Standard	2 mL	1 mL	0.5 mL

Best Practices

- Use calibrated pipettes and DNase-free pipette tips.
- Prepare the working solution fresh for each assay. Discard the solution after 24 hours.
- Ensure that 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 2 – 2000 ng total mass per tube.
- Label the top, not the sides of the assay tubes.

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 the dye and enhancer each with the assay buffer in a 1:100 ratio, e.g. 100 uL dye and 100 uL enhancer into 10 mL buffer. 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 200 ng/µL standards and 1 – 20 µL of unknown DNA samples to the respective tubes and mix well.
6. Incubate assay tubes at room temperature for 5 minutes.

Recommended Sample Volume

These recommendations ensure that sample concentrations are within the total mass detection limits of the assay. Total assay volume should remain 200 µL. Adjust working solution volume accordingly.

Initial Sample Concentration	Recommended Sample Volume
0.2 – 200 ng/µL	10 µL
0.1 – 2 ng/µL	20 µL
200 – 1000 ng/µL	2 µL

Initial Sample Concentration	Recommended Sample Volume
1000 – 4000 ng/µL	1 µL

Sample Measurement

1. Launch the Fluoro dsDNA app using a DeNovix Fluorometer.
2. Use the drop-down menu to select the correct LED source for the DeNovix dsDNA Broad Range Assay.
3. Select the preferred standard curve method (2 point standards supplied) and then choose Generate New Standard Curve.
4. Insert the 0 ng/µL dsDNA standard, lower the lid and tap **Measure**.
5. Insert the 200 ng/µL dsDNA 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 dsDNA Broad Range Dye (100x)	Yes	4°C - Room Temperature
DeNovix dsDNA Broad Range Buffer	Optional	4°C - Room Temperature
DeNovix dsDNA Broad Range Enhancer (100x)	Optional	4°C - Room Temperature
dsDNA Standards	Yes	4°C

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