DeNovix dsDNA Broad Range 2 Point Assay Instructions

Technical Note 142

Introduction

The DeNovix dsDNA Broad Range Assay enables 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 ng/µL to 2000 ng/µL when using 1 to 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.

Kit Contents

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

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)	0.5 mL	0.5 mL	0.5 mL
0 ng/µL dsDNA Standard	0.5 mL	0.5 mL	0.5 mL

Best Practices

- Use calibrated pipettes and DNase-free pipette tips.
- Prepare the working solution fresh for each assay.
- Protect the dye and working solutions from light.
- Ensure 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.

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 100 µL of the dye and 100 µL of the enhancer. 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.
   - Use thin-walled, clear UV-transparent 0.5 mL PCR tubes for assay measurements (DeNovix cat# TUBE-PCR-0.5-500 or equivalent). Label the top, not the sides of the assay tubes.
4. 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.
   - 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.
5. Incubate assay tubes at room temperature for 5 minutes. Protect from light.

Sample Measurement

1. Launch the Fluoro dsDNA app using a DeNovix fluorometer.
2. Use the drop-down menu to select the **DeNovix dsDNA Broad Range Assay**.
3. Select **Preconfigured 2 Standards** 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**.

Refer to Technical Note 143 available at www.denovix.com for additional information regarding reagent storage, solvent compatibility and multi-point standard curve instructions.

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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