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

KIT- DSDNA-BROAD-2 includes sufficient volume of the concentrated buffers and dsDNA standards to perform 1000 assays based on 200 µL volumes.

Component	Volume
DeNovix dsDNA Broad Range Dye (100x)	2 x 1 mL
DeNovix dsDNA Broad Range Buffer	250 mL
DeNovix dsDNA Broad Range Enhancer (100x)	2 x 1 mL
200 ng/µL dsDNA Standard (calf thymus)	0.5 mL
0 ng/µL dsDNA Standard	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.
- 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.
   - Avoid introducing air bubbles when mixing.
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.

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