DeNovix dsDNA High Sensitivity 2 Point Assay Instructions

Technical Note 144

Introduction

The DeNovix dsDNA High Sensitivity Assay enables accurate detection of purified double-stranded DNA (dsDNA) samples with a standard detection range from 100 pg to 250 ng total mass in 200 µL volumes. This equates to sample concentrations of 10 pg/µL to 250 ng/µL when using between 1-20 µL sample volumes in 200 µL assay volumes. The assay is linear for sample concentrations as high as 250 ng/µL when adjusting volumes to 1 µL of sample into 199 µL of working reagent. Total mass should not exceed 250 ng for best results.

The lower detection limit can be extended down to 5 pg/µL sample by adding 20 µL of the 5 pg/µL sample to 180 µL of the working reagent.

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 High Sensitivity Dye (100x)	2 x 1 mL	0.5 mL	100 µL
DeNovix dsDNA High Sensitivity Buffer	200 mL	50 mL	10 mL
25 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 properly calibrated pipettes and DNase-free pipette tips.
- Protect the dye and working solutions from light.
- Treat all standards and samples identically in terms of incubation times and temperature.
- Avoid introducing air bubbles when mixing.
- Generate a new standard curve for each assay.
- Ensure sample solution contaminant levels are compatible with the assay.

Sample Prep

1. Allow all solutions to equilibrate to room temperature before use. Vortex, then centrifuge vials briefly before opening 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. Scale volumes as needed to make enough volume to aliquot 190 µL of the mixture for each standard and unknown. Discard after 24 hours.
3. For each standard or unknown sample, add 190 µL of the working solution into a labeled tube. Adjust volume when adding more or less than 10 µL of the unknown sample.
   - Use thin-walled, clear 0.5 mL PCR tubes for assay measurements (DeNovix cat# TUBE-PCR-0.5-500 or equivalent). Label only the tops of the tubes.
4. Add 10 µL of the 0 ng/µL, 25 ng/µL standards or 1-20 µL of unknown DNA samples to the respective tubes and mix well. Avoid introducing air bubbles when mixing.
   - Assay total mass must be considered when deciding how much sample to use. This assay is appropriate for 100pg - 200ng total mass per tube.
5. Incubate assay tubes at room temperature for 5 minutes. Protect from light.

Sample Measurements

1. Launch the Fluoro dsDNA app using a DeNovix fluorometer.
2. Use the drop-down menu to select the **DeNovix dsDNA High Sensitivity Assay**.
3. Select **Preconfigured 2 Standards** and then choose **Generate New Standard Curve**.
4. Insert the 0 ng/µL dsDNA standard tube, lower the lid and tap the **Measure** button.
5. Insert the 25 ng/µL dsDNA standard tube, lower the lid and tap the **Measure** button.
6. After both standards are measured, tap the **Samples** button, insert a sample tube and tap **Measure.**

Refer to Technical Note 145 available at www.denovix.com for additional information regarding reagent storage, solvent compatibility and multi-point standard curve instructions.

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