

DeNovix dsDNA Ultra High Sensitivity 2 Point Assay Instructions

Technical Note 146

Introduction

The DeNovix dsDNA Ultra High Sensitivity Assay enables accurate detection of purified double-stranded DNA (dsDNA) samples with a detection range from 5 pg to 3 ng total mass per assay tube. This is equivalent to sample concentrations of 0.5 pg/ μ L to 300 pg/ μ L.

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 Ultra High Sensitivity Dye (400x)	0.5 mL	125 μ L	25 μ L
DeNovix dsDNA Ultra High Sensitivity Buffer	200 mL	50 mL	10 mL
DeNovix dsDNA B Ultra High Sensitivity Enhancer (100x)	2 x 1 mL	0.5 mL	100 μ L
300 pg/ μ L dsDNA Standard (calf thymus)	0.5 mL	0.5 mL	0.5 mL
0 pg/ μ L dsDNA Standard	0.5 mL	0.5 mL	0.5 mL

Best Practices

Pay careful attention to pipetting accuracy when quantitating low picogram amounts of dsDNA.

- Use properly calibrated pipettes and DNase-free pipette tips. Use the smallest calibrated pipettor available to dispense each sample volume.
- If sample dilutions are required, perform dilutions in the recommended assay tubes (DeNovix cat# TUBE-PCR-0.5-500 or equivalent).
- Prepare fresh working solution for each assay.
- Ensure all samples and standards are treated identically in terms of incubation times and temperature.

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 25 μ L of the dye and 100 μ L of the enhancer. Scale volumes as needed to make enough volume to aliquot 200 μ L of the mixture for each standard and unknown.
3. For each standard or unknown sample, add 200 μ L of the working solution into a labeled tube.
 - Use only thin-walled, clear 0.5 mL PCR tubes for assay measurements (DeNovix cat# TUBE-PCR-0.5-500 or equivalent). Label only the top, not the sides of an assay tube.
4. Add 10 μ L of the 0 pg/ μ L, 300 pg/ μ L standards or 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 5 pg to 3 ng 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 Ultra High Sensitivity Assay**.
3. Select **Preconfigured 2 Standards** and then choose **Generate New Standard Curve**.
4. Insert the 0 pg/ μ L dsDNA standard, lower the lid and tap **Measure**.
5. Insert the 300 pg/ μ L 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 147 available at www.denovix.com for additional information regarding reagent storage, solvent compatibility and multi-point standard curve instructions.

