Technical Note 146

Ultra High Sensitivity Assay Standard Protocol

Introduction

The DeNovix® dsDNA Ultra High Sensitivity Assay enables the 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 – 300 pg/µL.

Kit Contents

Kits are available in 1000, 250 and 50 (evaluation size) assays and include the components in Table 1.

Table 1: Ultra High Sensitivity Assay Kit Contents

Component	1000	250	EVAL
DeNovix dsDNA Ultra High Sensitivity Dye (400x)	0.5 mL	125 µL	25 µL
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 µL
300 pg/µL dsDNA Standard (calf thymus)	2 mL	1 mL	0.5 mL
0 pg/µL dsDNA Standard	2 mL	1 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 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 5 pg – 3 ng total mass per tube.

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 µL of the dye and 100 µL of the enhancer. Scale volumes as needed to make enough volume to aliquot 200 µL of the mixture for each standard and unknown.
3. For each standard or unknown sample, add 200 µL of the working solution into a labeled tube.
4. 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.
5. Add 10 µL of the 0 pg/µL, 300 pg/µL standards or unknown DNA samples to the respective tubes and mix well.
6. Incubate assay tubes at room temperature for 5 minutes.

Sample Measurement

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/µL dsDNA standard, lower the lid and tap Measure.
5. Insert the 300 pg/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 Ultra High Sensitivity Dye (400x)	Yes	4°C - Room Temperature
DeNovix dsDNA Ultra High Sensitivity Buffer	Optional	4°C - Room Temperature
DeNovix dsDNA UltraHigh Sensitivity Enhancer (100x)	Optional	4°C - Room Temperature
dsDNA Standards	Yes	4°C

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