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.

View Detailed Protocol

Kit Contents

Kits are available in 1000, 250 and 50 (evaluation size) assays and include the components in Table 1. Safety data sheets are available at denovix.com/sds.

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).
- Use fresh or properly stored working solutions.
- Ensure that all samples and standards are treated identically in terms of incubation times and temperature.
- Avoid introducing air bubbles when mixing.
- Working solution can be safely stored in a light-protected place for 24 hours at ambient temperature or 1 month at 4°C.
- 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 200 µL of working solution for each standard and sample to be tested by diluting the dye 1:400 in the assay buffer. Dilute the enhancer solution 1:100 into the dye/buffer mixture, e.g. Mix 25 uL dye, 100 uL enhancer, and 10 mL buffer.
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.

Recommended Sample Volume

These recommendations ensure that sample concentrations are within the total mass detection limits of the assay.

Initial Sample Concentration	Recommended Sample Volume
0.5 – 300 pg/µL	10 µL

Sample Measurement

1. Launch the Fluoro dsDNA app using a DeNovix Fluorometer.

2. Use the drop-down menu to select the correct LED source for the DeNovix dsDNA Ultra High Sensitivity Assay.
3. Select the preferred standard curve method (2 point standards supplied) 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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