Technical Note 147

Ultra High Sensitivity Assay Detailed 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.

The fluorescent dye used in the kit is highly selective for dsDNA and provides a more accurate DNA concentration measurement in the presence of contaminating single-stranded DNA (ssDNA) or RNA than does traditional absorbance-based methods.

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 below.

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

The dye is a potentially harmful chemical. Exercise universal laboratory safety precautions when handling the dye, and dispose of the dye as hazardous chemical waste according to your local regulations.

Instrument Compatibility

The DeNovix dsDNA High Sensitivity Quantitation Assay is designed for use with fluorometers or fluorescence plate readers equipped with excitation and emission filters for detecting green fluorescence. The unique spectral properties of the kit dye make it especially well-suited for use with instruments with blue LED excitation sources.

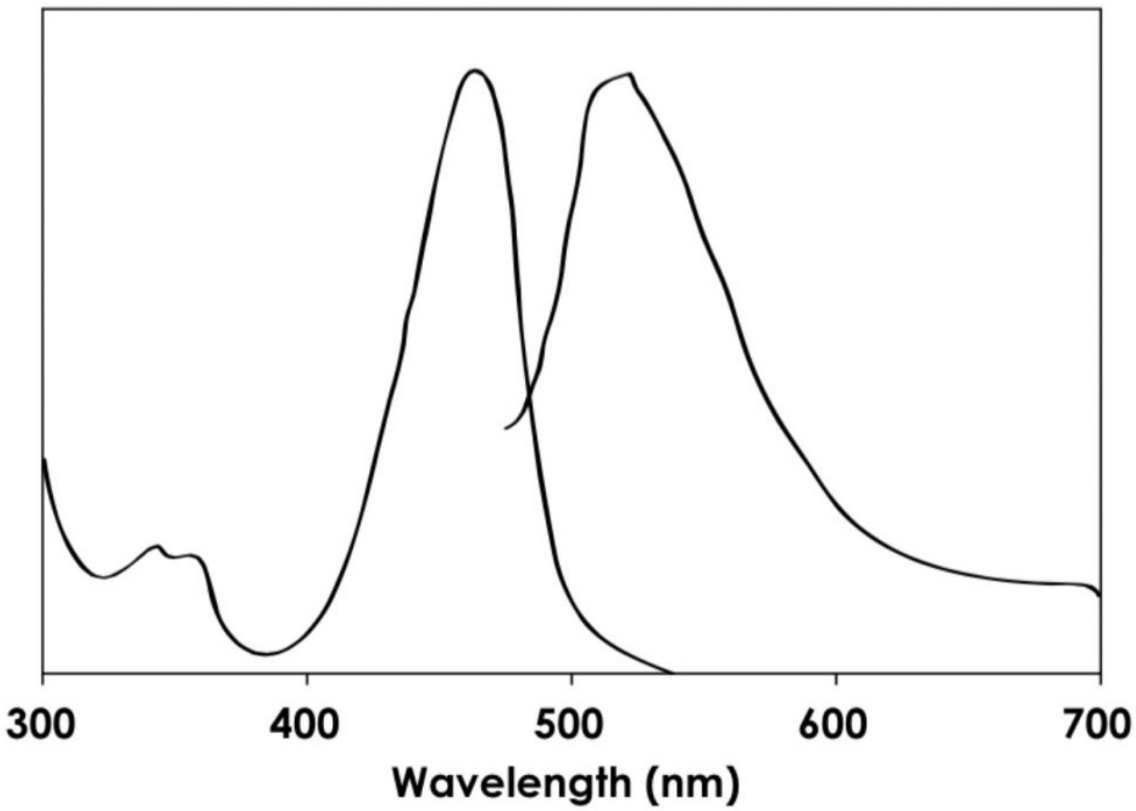

Figure 1: Excitation and emission spectra for DeNovix dsDNA Ultra High Sensitivity Range Quantitation Reagent in the presence of excess dsDNA.

Specific instructions using the 2 Point Standard Assay with DeNovix DS-11 FX, FX module or the QFX fluorometer are included in Technical Note 146.

Assay Considerations

Calf thymus DNA is provided as the reference standard as it is double-stranded, highly polymerized and is approximately 58% AT (42% GC). At times it may be preferable to use a dsDNA standard similar to the unknown samples (e.g. similar in size, linear vs circular). For bacterial DNA, consider using a species-specific standard, as the GC content varies widely depending on the species.

Although many instruments, including the DeNovix DS-11 FX and QFX Fluorometers, offer the option to use previously saved values, it is recommended that a new standard curve be generated at the time of the assay for optimal results.

Assay Linearity and Detection Limits

Fluorescent quantification specifications are often expressed in a variety of conventions. The linear range of this assay can be expressed in the following equivalent specifications:

Table 2: Ultra High Sensitivity Linearity and Detection

Specification	Range
Absolute mass per assay tube	5 pg to 3000 pg per 200 µL
Concentration in sample stock tube	0.5 pg/µL to 300 pg/µL

Note: These ranges are determined using the DeNovix FX Fluorometer. Your results may vary as a function of the sensitivity and accuracy of the instrument used.

Reagent Storage

The kit is stable for 12 months from ship date when stored as recommended.

Table 3: Ultra High Sensitivity Assay Reagent Storage

Component	Protect from Light	Temperature
DeNovix dsDNA Ultra High Sensitivity Dye (400x)	Yes	4°C - Room Temperature

Component	Protect from Light	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

Best Practices

It is important to pay careful attention to pipetting accuracy and overall sample handling techniques when quantitating picogram amounts of dsDNA.

- Use properly calibrated pipettes and DNase-free pipette tips. Use the smallest calibrated pipettor available to dispense each sample volume.
- Vortex or shake each component and centrifuge vials briefly before opening to minimize reagent loss on the cap.
- Use thin-walled, clear 0.5 mL PCR tubes for assay measurements (DeNovix cat #TUBE-PCR-0.5-500 or equivalent) or black-walled microplates. If using tubes, label only the top, not the sides of the tube.
- If sample dilutions are required to ensure that total mass does not exceed 3 ng per assay, perform all dilutions in the recommended assay tubes .
- Minimize assay tube and solution temperature fluctuations.
- Ensure that all samples and standards are treated identically in terms of incubation times and temperature.
- Although standard curves may be saved and re-used, it is recommended that a new curve is generated for each assay.
- Avoid introducing air bubbles into the sample solution when mixing samples.
- Ensure that all sample concentrations in the assay tubes or microplate wells fall within the limits of the reagent kit for accurate results.
- Use fresh or properly stored working solutions.
- Working solution can be safely stored in a light-protected place for 24 hours at ambient temperature or 1 month at 4°C.

Assay Protocol

1. Allow all solutions to equilibrate to room temperature before use.
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. Mix well before use.
3. For each standard or unknown sample, add 200 µL of the working solution per tube or micro well.
4. Add 10 µL of each standard or unknown DNA sample to the tube or micro well and mix well.
5. Avoid introducing air bubbles when mixing samples and working reagent.
6. Incubate standards and samples at room temperature for 5 minutes.
7. Generate the standard curve and then measure the samples using the proper excitation source and emission filters.

Standard Dilutions

Preparing diluted standards is not required when using the optimized preconfigured 2 Point Assay option in the DeNovix FX or QFX software. For the DeNovix User Defined Standards option or for use on microplate readers, prepare DNA standards by serial dilution of the 300 pg/µL standard in the 1X assay buffer as shown in the table below.

Standard	DNA	TE
300 pg/µL	100 µL of 300 pg/µL stock tube	None
150 pg/µL	100 µL of 300 pg/µL standard	100 µL
50 pg/µL	75 µL of 150 pg/µL standard	150 µL
10 pg/µL	40 µL of 50 pg/µL standard	160 µL
2 pg/µL	40 µL of10 pg/µL standard	160 µL
1 pg/µL	100 µL of 2 pg/µL standard	100 µL
0.5 pg/µL	100 µL of1 pg/µL standard	100 µL
0 pg/µL	100 µL of 0 ng/µL stock tube	None

Data Analysis

Sample concentrations are automatically calculated when using a DeNovix DS-11 FX or QFX Fluorometer.

For all other instruments, follow the instructions below:

1. Generate a standard curve to determine the unknown DNA concentration.

2. Average replicate values for each sample and subtract the average zero DNA value from each data point.
3. Plot the fluorescence RFU values for the DNA standards on the y-axis and pg/well DNA on the x-axis, and fit a trend line (Figure 2) through these points to generate a standard curve with a y-intercept = 0.
4. Use the equation for the trend line to calculate the amount of unknown DNA in each well (y = fluorescence and x = pg DNA per well or tube).

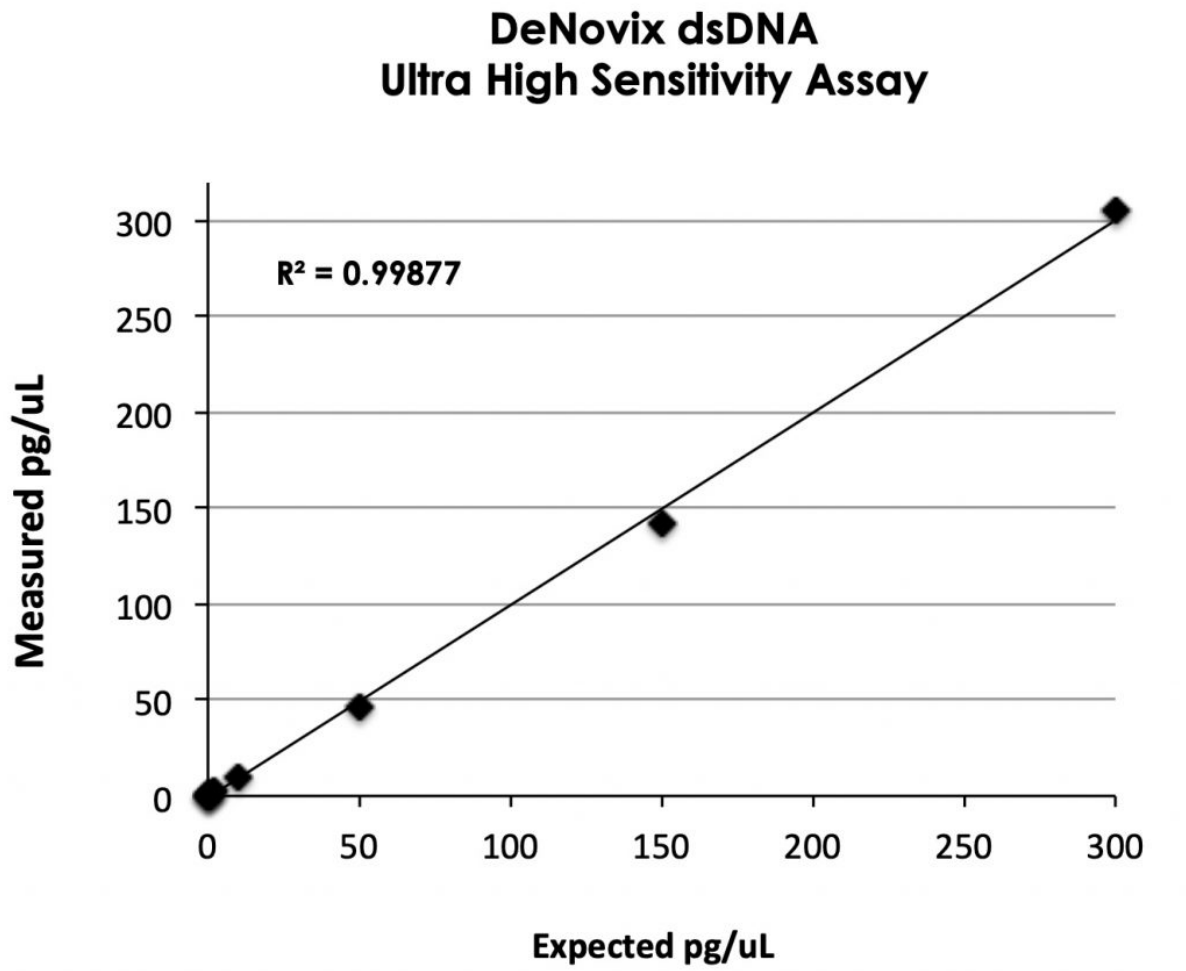

Figure 2: Standard range using 10 µL of sample in 200 uL of dye reagent.

Troubleshooting

- Confirm that the correct excitation source and emission filters were used at the time of the measurement. The DeNovix DS-11 and QFX software automatically uses the correct LED and emission filter.
- Confirm that standard concentrations and dilutions were performed correctly.
- Confirm that the correct concentration units for the standard curve and the unknown samples were used to calculate the stock concentrations.
- If applicable, ensure that the correct dilution factor or sample volume added value is entered into the appropriate Run screen field before a measurement is made.

Appendix: Solvent Compatibility

Table 5: Ultra High Sensitivity Assay Appendix

Compound	Maximum concentration in 200 µL assay	Signal Decrease (%)
Sodium Chloride	25 mM	0.17
Magnesium Chloride	5 mM	0.3
Sodium Acetate	30 mM	0.11
Ethanol	0.01	0.08
Phenol	0.001	0.1
Tween-20	5.0E-5	0.08
SDS	0.0001	0.87
SDS	1.0E-5	0.13

Compound	Maximum concentration in 200 µL assay	Signal Decrease (%)
Trtion X-100	0.0002	0.18
dNTPs*	100 µM	0.01

*DNA standards assayed in the absence or presence of contaminants listed at the concentrations above. *Mix of dATP, dCTP, dGTP, dTTP.*

DeNovix Assays

If the Ultra High Sensitivity Assay does not cover the concentration range of your samples, consider using an alternate DeNovix dsDNA Assay Kit.

For comparison, the standard detection ranges of the three assays are as follows:

Assay Detection Ranges

DeNovix dsDNA Assay	Range
Broad Range	0.1 – 2000 ng/µL
High Sensitivity	10 pg/µL – 250 ng/µL
Ultra High Sensitivity	0.5 – 300 pg/µL

Summary

Contact DeNovix Customer Support if further help is required. Outside of the US, please contact your local distributor for assistance.

For instructions specific on performing a 2 Point Standard Curve Assay on a DeNovix Fluorometer, refer to Technical Note 146 – Ultra High Sensitivity Assay Standard Protocol.

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