DeNovix dsDNA Broad Range Assay

Technical Note 143

Introduction

The DeNovix dsDNA Broad Range Assay enables accurate detection of purified double-stranded DNA (dsDNA) samples with a standard detection range from 2 to 2000 ng total mass in 200 µL volumes. This equates to sample concentrations of 0.1 ng/µL to 2000 ng/µL when using 1 to 20 µL sample volumes in a 200 µL total assay volume.

Extended Range

The upper detection limit can be extended to 4000 ng/µL by adding 1 µL of a 4000 ng/µL sample to 199 µL of working reagent.

- Note: There is some loss of linearity with this assay when adding more than 2000 ng total mass per assay tube.

Kit Contents

Item number KIT- DSDNA-BROAD-2 includes sufficient quantities of the concentrated buffers and calf thymus dsDNA standard to perform 1000 assays based on 200 µL measurement volumes.

Component	1000 assays
DeNovix dsDNA Broad Range Dye (100x)	2 x 1 mL
DeNovix dsDNA Broad Range Buffer	250 mL
DeNovix dsDNA Broad Range Enhancer (100x)	2 x 1 mL
200 ng/µL dsDNA Standard (calf thymus)	0.5 mL
0 ng/µL dsDNA Standard	0.5 mL

Store the kit at 4° C and protect the dye solution from light. The kit is stable for 6 months from date of receipt when stored as recommended.

Instrument Compatibility

The spectral properties of the dye are excitation/emission of 350/460 nm in the presence of dsDNA as shown in Figure 1 below:

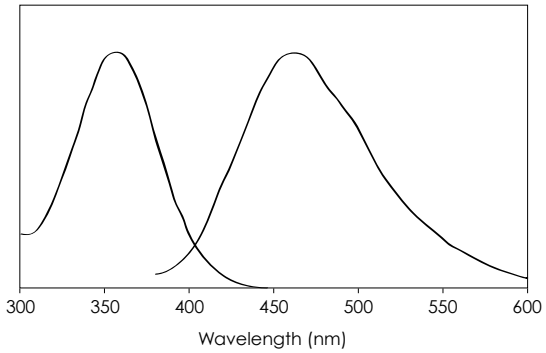

Fig. 1: Excitation and emission spectra for DeNovix dsDNA Broad Range quantitation reagent in the presence of excess dsDNA.

The kit is compatible with fluorescence microplate readers and fluorometers with the appropriate excitation sources and emission detectors.

Specific instructions (Technical Note 142) using the 2 point standard assay with DeNovix DS-11 FX , FX module or the QFX fluorometer are available at www.denovix.com.

Assay Considerations

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

Although many instruments including DeNovix DS-11 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 full detection range (including the extended range) of this assay can be expressed in the following specifications:

Specification	Range
Absolute mass per assay tube	2 ng to 2000 ng per 200 µL
Concentration in sample stock tube	100 pg/µL to 4000 ng/µL

Best Practices

- Prepare the working solution fresh for each assay. Discard the solution after 24 hours.
- Use properly calibrated pipettes and DNase-free pipette tips for best accuracy.
- Use thin-walled, clear UV compatible 0.5 mL PCR tubes (DeNovix Cat. No # TUBE-PCR-0.5-500 or equivalent) or black-walled 96 well microplates.
- Do not label the side of an assay tube as this could interfere with the sample measurement.
- Avoid introducing air bubbles into the sample solution when mixing samples.
- Minimize assay tube and solution temperature fluctuations.
- Ensure all samples and standards are treated identically in terms of incubation times and temperature.
- Ensure all sample concentrations in the assay tubes or microplate wells fall within the limits of the reagent kit.

Assay Prep

- Allow all solutions to equilibrate to room temperature before use.

  Note: The DeNovix dsDNA Broad Range dye is provided in DMSO, which may freeze during storage at 4°C.

- Vortex, then centrifuge vials briefly before opening to minimize reagent loss on the cap.

Assay Protocol

1. Prepare working solution by mixing 10 mL of the assay buffer with 100 µL of the dye and 100 µL of the enhancer.
   - Scale volumes as needed to make enough volume to aliquot 190 µL of the mixture per standard and unknown to be measured.
2. For each standard or unknown sample, add 190 µL of the working solution to a labeled tube or micro well. Adjust volume when adding more or less than 10 µL of the unknown sample.
3. Add 10 µL of the 0 ng/µL and 200 ng/µL standards and 1-20 µL of unknown DNA samples to the respective tubes and mix well.
4. Incubate standards and samples at room temperature for 5 minutes. Protect from light.
5. 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 200 ng/µL standard provided in 1X TE buffer (10 mM Tris pH 7-8, 1 mM EDTA).

Standard	DNA	TE
200 ng/µL	275 µL of 200 ng/µL stock tube	None
150 ng/µL	75 µL of 200 ng/µL standard	25 µL
100 ng/µL	100 µL of 200 ng/µL standard	100 µL
25 ng/µL	50 µL of 100 ng/µL standard	150 µL
12.5 ng/µL	100 µL of 25 ng/µL standard	100 µL
6.25 ng/µL	100 µL of 12.5 ng/µL standard	100 µL
2 ng/µL	32 µL of 6.25 ng/µL standard	68 µL
0 ng/µL	None	100 µL

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 replicates 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 ng/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 = ng DNA per well or tube).

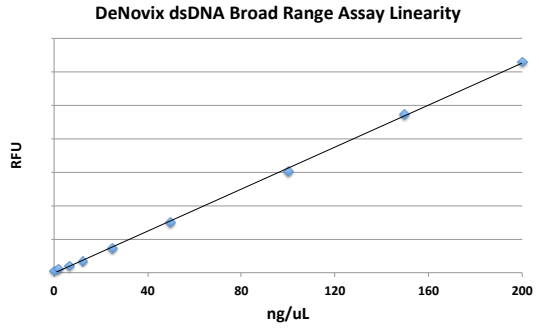

Fig. 2. Calf Thymus DNA measured using the DeNovix dsDNA Broad Range Assay on a DS-11 FX fluorometer.

Solvent Compatibility

Compound	Final maximum concentration in assay (200 µL)
Ammonium Acetate	5 mM
Sodium Chloride	50 mM
Ethanol	0.5%
Phenol	0.1%
SDS	0.01%
dNTPs*	100 µM

Troubleshooting

- Review the Best Practices recommendations.
- Confirm tubes or assay plates are UV transparent,
- Confirm that the correct excitation source and emission filters were used at the time of the measurement.
  - Note: The DeNovix DS-11 and QFX software automatically uses the correct LED and emission filter.
- Confirm that standard concentrations and dilutions are performed correctly.
- Confirm that the correct concentration units for the standard curve and the unknown samples are 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.

DeNovix Assays

If the Broad Range 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:

DeNovix dsDNA Assay	Range
Broad Range	100 pg/µL to 2000 ng/µL
High Sensitivity	10 pg/µL to 250 ng/µL
Ultra High Sensitivity	0.5 pg/µL to 300 pg/µL

Instructions specific to performing a 2 Point standard curve assay on a DeNovix fluorometer (Technical note 142) is available at www.denovix.com

Customer Support

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

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