Technical Note 143

Broad Range Assay Detailed Protocol

Introduction

The DeNovix dsDNA Broad Range Assay enables the 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 – 2000 ng/µL when using 1 – 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

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. Safety data sheets are available at denovix.com/sds.

Table 1: dsDNA Broad Range Assay Kit Contents

Component	1000	250	EVAL
DeNovix dsDNA Broad Range Dye (100x)	2 x 1 mL	0.5 mL	100 µL
DeNovix dsDNA Broad Range Buffer	250 mL	50 mL	10 mL
DeNovix dsDNA Broad Range Enhancer (100x)	2 x 1 mL	0.5 mL	100 µL
200 ng/µL dsDNA Standard (calf thymus)	2 mL	1 mL	0.5 mL
0 ng/µL dsDNA Standard	2 mL	1 mL	0.5 mL

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.

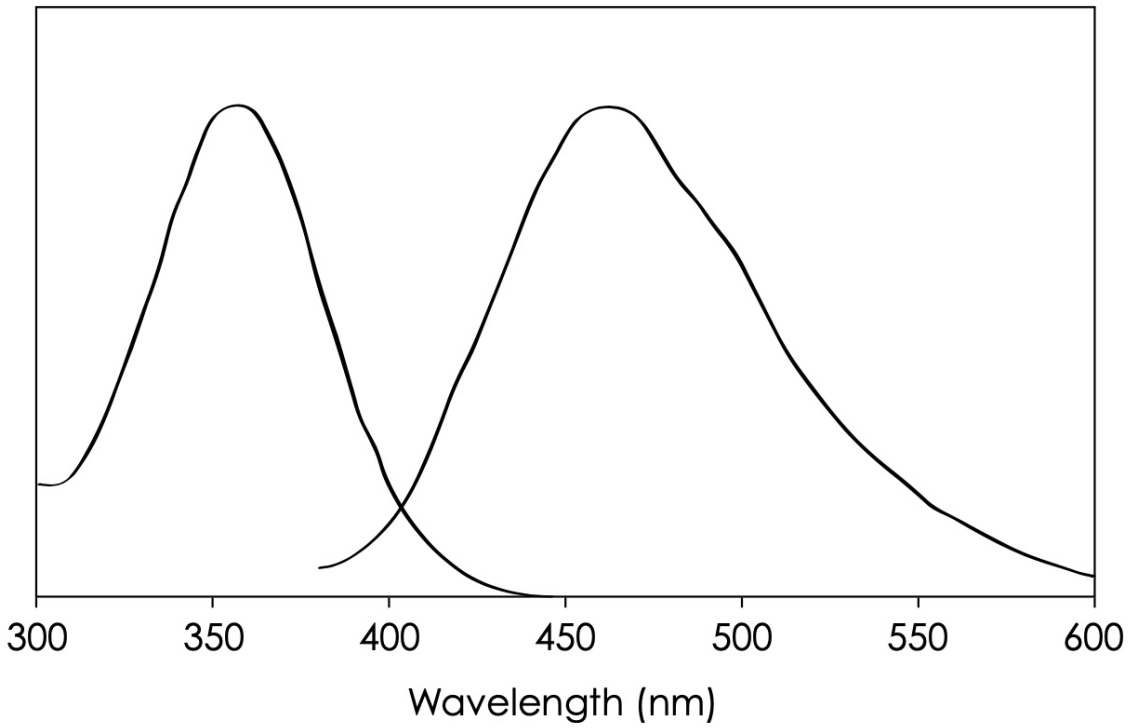

Figure 1: Excitation and emission spectra for DeNovix dsDNA Broad Range Quantitation Reagent in the presence of excess dsDNA.

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

Specific instructions using the 2 Point Standard Assay with DeNovix DS-11 FX, FX module or the QFX fluorometer are included in Technical Note 142.

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 alternatively dsDNA standard more similar (e.g., 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:

Table 2: Broad Range Assay Linearity and Detection Limits

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

Reagent Storage

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

Table 3: Broad Range Assay Reagant Storage

Component	Protect from Light	Temperature
DeNovix dsDNA Broad Range Dye (100x)*	Yes	4°C - Room Temperature
DeNovix dsDNA Broad Range Buffer	Optional	4°C - Room Temperature
DeNovix dsDNA Broad Range Enhancer (100x)	Optional	4°C - Room Temperature
dsDNA Standards	Yes	4°C

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

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 #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 that all samples and standards are treated identically in terms of incubation times and temperature.

- Ensure that all sample concentrations in the assay tubes or microplate wells fall within the limits of the reagent kit.

Assay Protocol

1. Allow all solutions to equilibrate to room temperature before use.
2. Vortex, then centrifuge vials briefly before opening to minimize reagent loss on the cap.
3. Prepare working solution by mixing the dye and enhancer each with the assay buffer in a 1:100 ratio, e.g. 100 uL dye and 100 uL enhancer into 10 mL buffer.
4. Scale volumes as needed to make enough volume to aliquot 190 µL of the mixture per standard and unknown to be measured.
5. 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.
6. 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.
7. Incubate standards and samples at room temperature for 5 minutes.
8. Generate the standard curve and then measure the samples using the proper excitation source and emission filters.

Recommended Sample Volume

These recommendations ensure that sample concentrations are within the total mass detection limits of the assay. Total assay volume should remain 200 µL. Adjust working solution volume accordingly.

Initial Sample Concentration	Recommended Sample Volume
0.2 – 200 ng/µL	10 µL
0.1 – 2 ng/µL	20 µL
200 – 1000 ng/µL	2 µL
1000 – 4000 ng/µL	1 µL

Standard Dilutions

Preparing diluted standards is not required when using the 2 point assay option supplied. 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).

Table 4: Broad Range Assay Standard Dilutions

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).

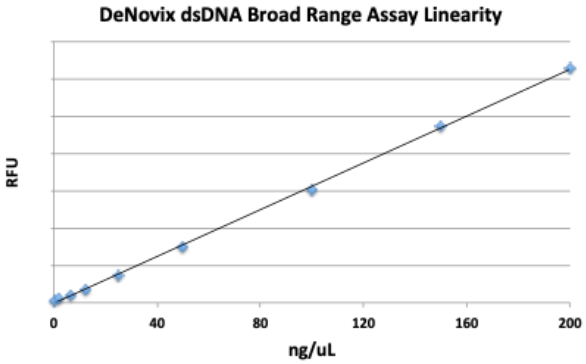

Figure 2: Calf Thymus DNA measured using the DeNovix dsDNA Broad Range Assay on a DS-11 FX Fluorometer.

Solvent Compatibility

Table 5. The tolerance of the dye in the presence of common solvents while maintaining linearity.

Compound	Final concentration in assay (200 uL)	Signal Decrease (%)
Sodium Chloride	25 mM	-5%
Sodium Acetate	30 mM	50%
Magnesium Chloride	5 mM	35%
SDS	0.01%	32%
SDS	0.001%	-5%
Ethanol	1%	4%
Phenol	0.10%	3%
Triton X-100	0.01%	8%
Triton X-100	0.001%	-5%
Tween-20	0.005%	20%
CTAB*	0.0005%	64%
dNTPs	100 uM	-1%
BSA**	0.05 mg/mL	27%

** Average change for 250 ng to 2000 ng samples. Complete loss of signal is seen below 250 ng.*
*** Average change for 20 ng to 2000 ng samples. Not compatible with quantitation below 20 ng due to increased background at low DNA concentrations*

Troubleshooting

- Review the Best Practices recommendations.
- Confirm that 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 seen in Table 6.

Table 6: DeNovix Assays

Assay Detection Ranges

DeNovix dsDNA Assay	Range
Broad Range	0.1 – 2000 ng/µL (extended range to 4000 ng/uL)
High Sensitivity	10 pg/µL – 250 ng/µL (extended range down to 5 pg/uL)
Ultra High Sensitivity	0.5 – 300 pg/µL

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