DeNovix®

Fluorescence Quantification of Nucleic Acid and Proteins

Best Practices

Introduction

Fluorometric quantitation assays for nucleic acids and protein are now routine in many life science research labs. As with many fluorescence techniques, it is important to apply best practices to ensure reliable results and reduce user error.

This document provides some best practice tips for making fluorescent measurements using the DeNovix DS-11 Series.

LED Selection

Ensure that the excitation source (LED) and emission filters are appropriate for the fluorophore or assay of interest (Table 1). DeNovix Fluorescence instruments are equipped with four user-selectable LED light sources. See Figure 1 for a list of common fluorophores.

LEDs	Excitation Filter Range	Emission Filter Range
UV (375 nm)	361 – 389 nm	435 – 514 nm
BLUE (470 nm)	442 – 497 nm	514 – 567 nm
GREEN (525 nm)	490 – 558 nm	565 – 650 nm
RED (635 nm)	613 – 662 nm	664 – 740 nm

Sample Tubes

- Use only thin-walled, clear 0.5 mL PCR tubes for sample measurements.
- Use 200 µL final volumes for all standards and samples.
- Do not label the side of an assay tube, as this could interfere with the sample measurement.
- Ensure that the tube is clean and dry on the outside before inserting into the DS-11 Series tube chamber. Moisture and condensation on the tube surface may lead to measurement errors.

Sample Preparation

- Protect dye reagents and working solutions from light.
- Confirm that fluorophore assay reagent is compatible with sample buffer components.
- Follow assay manufacturer's recommendations regarding standard curve concentrations.
- Treat all samples and standards identically in terms of volumes, incubation times and temperature.
- Measure all standards and samples within the assay manufacturer's recommended assay time frames.

Sample Measurements

- Ensure that the assay tubes (and solutions) are at room temperature at the time of the measurement. Temperature fluctuations may impact the accuracy of the assay.
- Ensure that sample solutions are homogeneous and well-mixed before sampling. Avoid introducing air bubbles into the sample solution when mixing sample.
- Close the fluorometer chamber lid before tapping the Measure button.

Figure 1: Common Fluorophores

UV

DeNovix dsDNA Broad Range Assay
Hoechst 33258

Blue

DeNovix dsDNA High Sensitivity Assay
DeNovix dsDNA Ultra High Sensitivity Assay
Qubit™ dsDNA HS assay
Qubit™ dsDNA BR assay
Quant-iT™ Protein
Qubit™ Protein
Quant-iT™ PicoGreen™
Quant-iT™ RiboGreen™
Fluorescein
FITC
Alexa Fluor™ 488
SYBR™ Green
GFP
Qubit™ ssDNA
Quant-iT™ dsDNA HS
Quant-iT™ dsDNA BR
QuantiFluor™ dsDNA
QuantiFluor™ One dsDNA
QuantiFluor™ RNA
Quant-iT™ microRNA
Qubit™ MicroRNA
Quant-iT™ OliGreen™
QuantiFluor™ ssDNA

Green

Alexa Fluor™ 555
Cy3
RFP

Red

DeNovix RNA Assay
Qubit™ RNA HS assay
Qubit™ RNA BR assay
Alexa Fluor™ 647
Quant-iT™ RNA BR assay
Quant-iT™ RNA HS assay
Cy5

Fluorescence Quantification Video

Learn how and when Fluorescence Quantification can be the better method to quantify biomolecules when using the DS-11 Series or QFX Fluorometer.

Note: Fluorescence results are reported in relative fluorescence units (RFU). Differences in RFU between different fluorometers are expected. Use a standard curve for accurate quantitation.