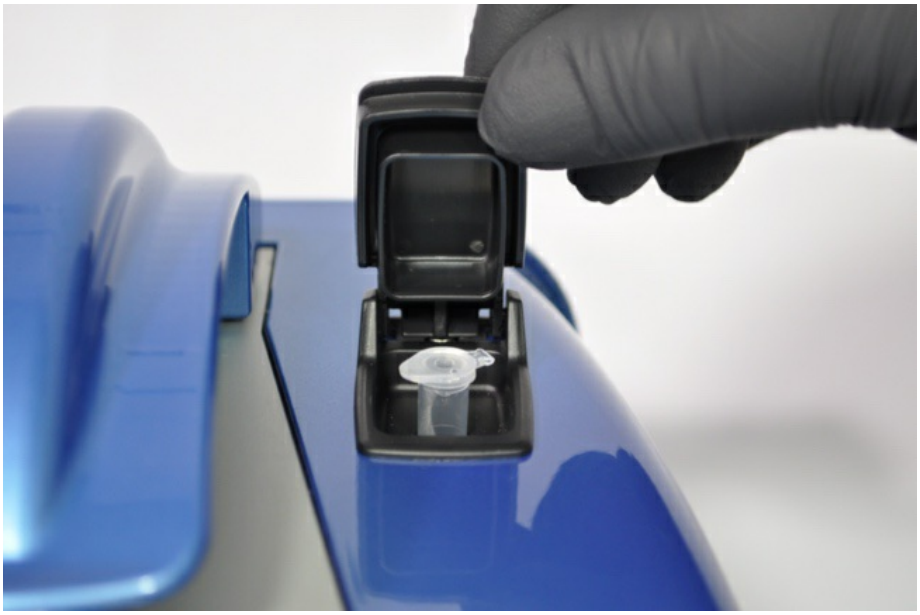
Technical Note 154

Fluorescence Measurement 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

Preconfigured assays automatically use the correct excitation sources and emission filters. For user-defined methods, ensure that the excitation source (LED) and emission filters are appropriate for the fluorophore or assay of interest (Table 1).

Table 1: Excitation and Emission Filter Ranges

LEDs	Excitation Filter Range	Emission Filter Range
UV	361 – 389 nm	435 – 514 nm
BLUE	442 – 497 nm	514 – 567 nm
GREEN	490 – 558 nm	565 – 650 nm
RED	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 homogenous and well-mixed before sampling. Avoid introducing air bubbles into the sample solution when mixing sample.
- Lower the DS-11 Series cover before tapping the Measure button.

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.

Revised 19 Oct 2020

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