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Quant-IT DNA Quantification (Assay)

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Perform dsDNA quantification easily and quickly with Quant-iT dsDNA assay kits. Both the Quant-iT High-Sensitivity dsDNA Assay Kit and the Quant-iT Broad-Range dsDNA Assay Kit provide concentrated assay reagent, dilution buffer, and pre-diluted DNA standards. These DNA assay kits are highly selective for double-stranded DNA over RNA, and, in the ranges of 0.2–100 ng DNA (for the HS dsDNA Assay Kit) and 2–1000 ng DNA (for the BR dsDNA Assay Kit), the fluorescence signal is linear.

Quant_iT_dsDNA_HS_Assay_UG.pdf

Quant_iT_dsDNA_BR_Assay_UG.pdf

Allyson Hirsch, George Testo 2022. Quant-IT DNA Quantification (Assay). **protocols.io** https://protocols.io/view/quant-it-dna-quantification-assay-cakbscsn

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The Quant-iT dsDNA High-Sensitivity Assay Kit and the Quant-iT dsDNA Broad-Range Assay Kit make DNA quantification easy and accurate. The kits provide concentrated assay reagent, dilution buffer, and prediluted DNA standards. Simply dilute the reagent 1:200, load 200 µL into the wells of a microplate, add 1–20 µL sample, mix, then read the fluorescence.

The assays are highly selective for double-stranded DNA over RNA, and, in the range of 0.2–100 ng for the HS assay or 2–1000 ng for the BR assay, the fluorescence signal is linear with DNA. The assays are performed at room temperature, and the signal is stable for three hours. Common contaminants, such as salts, solvents, detergents, and protein are well tolerated in these assays.

Reagents

- Quant-IT Reagent
- Quant-IT Buffer

Supplies

- 96 Well Black Greiner plate(s)
- Green temporary seal(s)
- Foil seal(s)

Equipment

- 10mL serological pipette & tip(s)
- 200uL multi-channel pipette & tips
- 20uL multi-channel pipette & tips
- 20uL pipette & tips

Exercise caution and make sure to use the appropriate PPE (lab coat, gloves, and safety precautions). Some reagents used for this procedure are potentially mutagenic. Dispose of all reagents appropriately.

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Making Working Solution

1 Make working solution (number of samples + 24 standards + 10% = total volume).

1.1 200uL Buffer (per sample) + 1uL Reagent (per sample)

> **Note:** Be sure to vortex buffer and reagent prior to adding to conical.

1.2 Cover conical with a foil seal to prevent light exposure.

1.3 

Vortex working solution.

Loading Working Solution

2 Using a 200uL 12-channel pipette and boat / reservoirs, load **200 µL** of the working solution into each sample and standard well on the microplate reader plate.

2.1 Cover working solution with a foil seal in between uses.

Loading Samples

3 

Centrifuge the sample plate(s).

4 Add **1-20 µL** of DNA from the sample plate(s) to each well on the microplate reader plate.

> **Note:** While duplicates and triplicates are suggested, it is not necessary.

Loading Standards

5 Add 10uL of each gamma DNA standard to each well on the microplate reader plate.

> **Note:** Duplicates or triplicates are recommended. When ordering the standards, do it in ascending order

6 Mix sample and standard wells using a 200uL 12-channel pipette by pipetting up and down gently.

OR: Use a plate microplate vortexer on a gentle setting such as **1200 rpm** .

Measuring Fluorescence

7 Cover plate(s) with a foil seal and carefully carry them to a microplate reader.

8

Measure fluorescence (FL 485/528nm) using a microplate reader.

8.1 Set microplate type as: 96 Well Greiner Plate or other equivalent.

> **Note:** Plate type can impact fluorescence values if not correctly specified during setup.

8.2 Export fluorescence table into an Excel document for future reference.

9 Use a standard curve to determine the concentration of DNA in ng/uL.

> **Note:** Revise standard curve calculations based on the amount of DNA quantified for

each sample.