

DNA QUANTIFICATION ON THE FLUOROMETER

The fluorometer used by our lab is the DyNA Quant 200 from Hoefer.

The following instructions are for this specific fluorometer.

We use the DyNA Quant protocol for Low Range (10-500 ng/ml final DNA Concentration)

The DyNA Quant Should NOT BE USED for supercoiled plasmid DNA, it will underestimate the concentration because supercoiling interferes with dye intercalation. Use the green machine spec instead.

The DyNA Quant should ALWAYS be used for genomic DNA, it will give a much more accurate reading than the spectrophotometer, which will tend to over-estimate due to presence of impurities in the sample.

Solutions used in the assay

Hoechst dye stock solution

Add 10 ml of sterile milli-Q water to 10 mg of Hoechst H 33258 dye.

Do not filter. Store at 4 C for up to 6 months in an amber bottle

10X TNE buffer (1000 ml, buffer stock solution)

In ~800 ml of sterile milli-Q water dissolve

12.11 g Tris (100 mM final concentration)

3.72 g EDTA Na₂-2H₂O (10 mM final concentraion)

116.89 g NaCl (2 M final concentraion)

Adjust pH to 7.4 with concentrated HCl. Add sterile distilled water to 1000 ml.

Filter before use (0.45 micron) without addition of the Hoechst dye

Store at 4 c for up to 3 months

DNA Standard

Calf Thymus DNA Stock (diluted to 1 mg/ml with 10X TNE buffer)

100 µl of 1 mg/ml Calf Thymus DNA Stock

100 µl 10X TNE buffer

800 µl sterile distilled water

Assay Solution

10 µl Hoechst H 33258 Dye Stock Solution

10 ml 10X TNE buffer

90 ml sterile distilled water

IMPORTANT

-Hoechst dye is a possible mutagen!

Wear gloves when handling and wear a mask when weighing.

-All solutions must be at room temperature before measuring fluorescence.

-Prepare Assay Solution fresh daily.

The Assay

Turn the instrument "ON" 15 minutes before use.

Zero the instrument

Prepare an assay blank using 2 ml of the *Assay Solution*

into the special cuvette. Wipe the sides and bottom of the cuvette with a low-lint tissue. Insert the cuvette, close the lid and press ZERO. After "0" displays, remove the cuvette

Calibrate the instrument

Deliver 2 μl of the *DNA Standard* into the 2 ml *Assay Solution* already in the cuvette. Mix by pipetting several times into a disposable transfer pipet. Place the cuvette in the well, close the lid and press CALIB. Enter 100 for the concentration in the cuvette.

Amount ($2 \mu\text{l} \times 100 \text{ ng}/\mu\text{l} = 200 \text{ ng}$);

Final conc, in cuvette $200 \text{ ng}/2\text{ml} = 100\text{ng/ml}$

Press ENTER. After the entered value is displayed remove the cuvette

Zero the Instrument

Empty the contents of the cuvette into a hazardous waste container. Drain the rinse water into the hazardous waste container also. Dry by draining the cuvette and blotting upside down on a paper towel.

Add 2 ml of the same *Assay Solution* as used above, insert the cuvette into the well, close the lid. Press ZERO. After "0" displays remove the cuvette

Measure the Sample

Add 2 μl of the sample and mix well. Place the cuvette in the well, close the lid and record the measurement.

Measure Additional Samples by zeroing the instrument and measuring the sample as listed above.

IMPORTANT NOTES:

Some labs dilute their Calf Thymus DNA to a different concentration than 1 mg/ml. This will dictate the value entered into the machine during the calibration.

Some labs do not zero the instrument after each reading. They calibrate the instrument with the calf thymus and record the reading. The 2 ul of sample is added to the calibration solution in the cuvette, mixed and a reading is obtained from which they subtract the previous reading. This addition and subtraction continues for as many samples as they have.