DNA quantification using the Qubit fluorometer

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1 Works for me

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ABSTRACT

Instructions to quantify double stranded DNA on a Qubit Fluorometer using the high sensitivity dsDNA assay kit

MATERIALS

NAME	CATALOG #	VENDOR
Qubit™ Assay Tubes	Q32856	Invitrogen - Thermo Fisher
Qubit 1X dsDNA High Sensitivity Assay Kit	Q33230	Thermo Fisher Scientific
Qubit™ 3 Fluorometer	Q33216	Thermo Fisher

MATERIALS TEXT

Different versions of the Qubit fluorometer may be used (check that the kit is compatible with your version) but set-up instructions may differ slightly

1 Prepare a mastermix of Qubit™ working solution for the required number of samples and standards. The Qubit dsDNA kit requires 2 standards for calibration (see note below).

Per sample:

Qubit® dsDNA HS Reagent **1 µl**

Qubit® dsDNA HS Buffer **199 µl**

If you have already performed a calibration on the Qubit machine for the selected assay you can use the previous calibration stored on the machine. We recommend performing a new calibration for every sample batch but a same-day calibration would be fine to use for multiple batches.

To avoid any cross-contamination, we recommend that you remove the total amount of working solution required for your samples and standards from the working solution bottle and then add the required volume to the appropriate tubes instead of pipetting directly from the bottle to each tube.

2 Label the tube lids. Do not label the side of the tube as this could interfere with the sample reading.

Use only thin-wall, clear, 0.5mL PCR tubes. Acceptable tubes include Qubit™ assay tubes (Cat. No. Q32856)

3 Aliquot Qubit™ working solution to each tube:
- standard tubes requires 190µL of Qubit™ working solution
- sample tubes require anywhere from 180–199µL (depending how much sample you wish to add).

The final volume in each tube must be 200µL once sample/standard has been added.

4 Add 10µL of standard to the appropriate tube.

5 Add 1–20µL of each user sample to the appropriate tube.

If you are adding 1–2µL of sample, use a P-2 pipette for best results.

6 Mix each tube vigorously by vortexing for 3–5 seconds.

7 Allow all tubes to incubate at room temperature for 2 minutes, then proceed to “Read standards and samples”.

8 On the Home screen of the Qubit™ 3 Fluorometer, press DNA, then select 1X dsDNA HS as the assay type. The Read standards screen is displayed. Press Read Standards to proceed.

If you have already performed a calibration for the selected assay, the instrument prompts you to choose between reading new standards and running samples using the previous calibration. **If you want to use the previous calibration, skip to step 12**. Otherwise, continue with step 9.

9 Insert the tube containing Standard #1 into the sample chamber, close the lid, then press Read standard. When the reading is complete (~3 seconds), remove Standard #1.

10 Insert the tube containing Standard #2 into the sample chamber, close the lid, then press Read standard. When the reading is complete, remove Standard #2.

11 The instrument displays the results on the Read standard screen. For information on interpreting the calibration results, refer to the Qubit™ Fluorometer User Guide, available for download at thermofisher.com/qubit.

12 Press Run samples.

13 On the assay screen, select the sample volume and units:
- Press the + or – buttons on the wheel, or anywhere on the wheel itself, to select the sample volume added to the assay tube (from 1–20µL).
- From the unit dropdown menu, select the units for the output sample concentration (in this case choose ng/µL).

14 Insert a sample tube into the sample chamber, close the lid, then press Read tube. When the reading is complete (~3 seconds), remove the sample tube.

15 **The top value (in large font) is the concentration of the original sample and the bottom value is the dilution concentration**. For information on interpreting the sample results, refer to the Qubit™ Fluorometer User Guide.

16 Repeat step 14 until all samples have been read.

17 Carefully **record all results** and store run file from the Qubit on a memory stick.

18 All negative controls should ideally be 'too low' to read on the Qubit machine, but MUST be < 1ng per ul. If your negative controls >1ng per ul, considerable contamination has occurred and you must redo previous steps.