Quantification of extracted DNA using the Quantus fluorometer (Promega) with QuantiFluor® Double stranded DNA (dsDNA) system

Promega

Abstract

Extracted DNA is quantified using the Quantus fluorometer (Promega) with QuantiFluor®

Double stranded DNA (dsDNA) system according to the instructions in the user manual. The QuantiFluor® dsDNA System contains a fluorescent DNA binding dye which enables sensitive and specific quantification of small amounts of dsDNA.

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Protocol

Calibration of the Quantus fluorometer

Step 1.

Prepare the QuantiFluor® dsDNA working solution by diluting the dye at a 1:200 dilution in 1X TE buffer (eg. 10µl of QuantiFluor® dsDNA dye to 1990µl of 1X TE buffer) in a empty 0.5ml PCR tube. Then mix the solution by pulse vortexing for 15 seconds

Step 2.

Prepare the Blank solution for calibration by adding and mixing 100µl of QuantiFluor® dsDNA dye working solution and 100µl of 1X TE buffer to an empty 0.5ml PCR tube

Step 3.

Prepare the standard sample by adding and mixing of 2µl of provided DNA standard to 98µl of 1X TE buffer in a empty 0.5ml PCR tube. Then add 100µl from QuantiFluor® dsDNA dye working solution and mix

Step 4.

Calibrate the Quantus fluorometer by reading the blank and standard sample

Measure an unknown sample

Step 5.

Dilute 1µl of the unknown sample in a 99µl of 1X TE buffer in a empty 0.5ml PCR tube. Then mix the solution by pulse vortexing for 15 seconds

Step 6.

Add 100µl of QuantiFluor® dsDNA dye working solution to the same 0.5ml PCR tube for a total volume of 200µl for quantification. Then mix the solution by pulse vortexing for 15 seconds

Step 7.

Incubate the mixed solution at the room temperature for 5 minutes protected from light

Step 8.

Set the Quantus fluorometer to dsDNA protocol and select the volume of the unknown sample (1µl) and the desired concentration units. Then the concentration of unknown sample is measured