Dec 05, 2017

Direct Double-Stranded DNA Quantitation from PCR Reactions V.2

Michael Van Dyke[1]

[1]Department of Chemistry and Biochemistry, Kennesaw State University

Working dx.doi.org/10.17504/protocols.io.k5pcy5n

Michael Van Dyke
Department of Chemistry and Biochemistry, Kennesaw State Uni...

ABSTRACT

For convenience and for PCR products that are challenging to purify with high efficiency (*e.g.*, chemically modified DNAs), it is often desirable to quantitate synthesized DNA directly from a PCR reaction. Here we describe the use of a high-sensitivity Quant-iT™ PicoGreen® dye-based fluorescence assay to quantitate PCR-synthesized, double-stranded, low molecular weight, 5'-modified DNA probes in the presence of single-stranded primers and deoxyribonucleotides.[1]

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Iain D. Johnson, 8.3 Nucleic Acid Quantitation in Solution, in *The Molecular Probes Handbook: A Guide to Fluorescent Probes and Labeling Technologies*, 11th Edition, Life Technologies Corporation, 2010, pp. 339-348.

GUIDELINES

Protocol development supported by grants from the NIH (1R15GM104833-01) and NSF (MCB-1714778).

MATERIALS

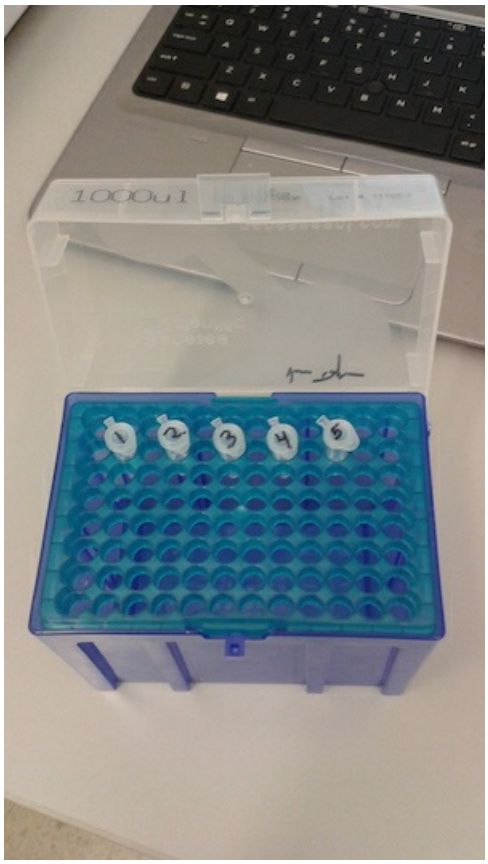
NAME	CATALOG #	VENDOR
Qubit™ dsDNA HS Assay Kit	Q32851	Invitrogen - Thermo Fisher
Axygen 0.5 mL PCR tubes, 0.5 mL, thin wall, clear, flat caps	PCR-05-C	Corning

Prepare Quant-iT™ dsDNA High-Sensitivity Working Reagent [WR1]

1 Dilute Quant-iT™ dsDNA HS reagent 1:200 with Quant-iT™ dsDNA buffer to a final volume of 200 µL * 1.1 * (total number of assays to be performed). For example, to perform 10 assays, prepare 1100 µL WR1. Prepare in polypropylene microcentrifuge tube, vortex briefly to mix thoroughly. Store shielded from direct light, room temperature. Use within 3 h preparation. Note: it is possible to substitute water for Quant-iT™ dsDNA buffer in the preparation of WR1. However, measured values for dsDNA will be ~33% lower than those obtained with Quant-iT™ dsDNA buffer.

Prepare assay samples

2 Label high-clarity 0.5 mL polypropylene microcentrifuge tubes on their caps. Axygen thin-wall PCR tubes (PCR-05-C) work well. Include blank if desired. Place tubes in a rack. A used 1000 µL pipet tip rack works well for this purpose.

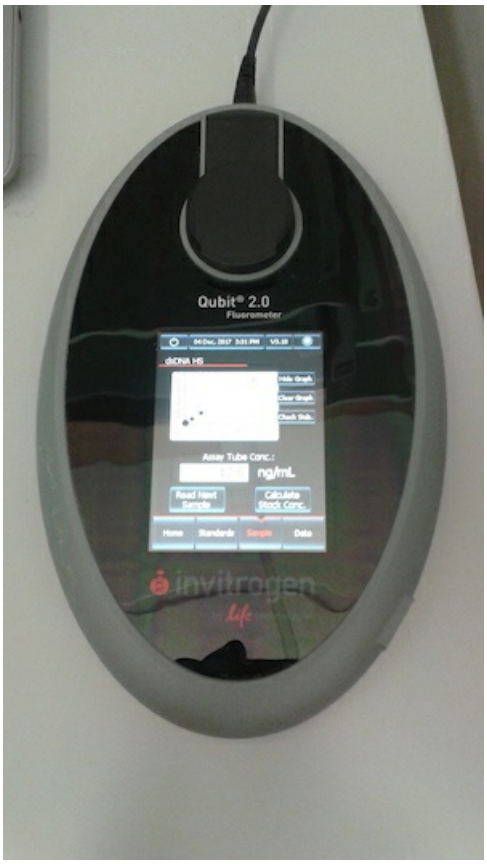


3 Aliquot 200 µL WR into each tube. Add 2 µL aliquot from completed PCR reaction. Vortex to mix thoroughly; centrifuge briefly to coalesce. Return tubes to rack. Incubate at room temperature for at least 5 min. Fluorescence values increase slightly with time (≤ 10%) and are stable for at least 1 h.

Read assay samples

4 Following instructions are for a Qubit™ 2.0 fluorometer. Adjust accordingly for fluorometer being used. Note that the Quant-iT™ dsDNA HS reagent fluorescence excitation maximum is 502 nm (blue) and emission maximum is 523 nm (green).

5 Turn on Qubit 2.0 fluorometer. Using touchscreen, “Choose Your Assay: DNA”, “dsDNA High Sensitivity”. “Read New Standards?” If desired “Yes”, otherwise “No”. Insert sample tube, close lid, “Read Next Sample”. Record data by hand or upload upon completion to a USB flash drive.



6 Note that the values shown are those for dilution in WR1; units are ng/mL. To determine double-stranded DNA concentration in PCR reaction, multiply values by 100 and convert to ng/µL. For PCR reactions with New England Biolabs *Taq* DNA polymerase and standard *Taq* buffer, synthesizing short 63-bp IRD7-labeled REPSA selection templates, we routinely obtain yields of 3 to 6 ng/µL product dsDNA. Blank samples without any added DNA template, whether stored on ice or processed through 20 PCR cycles, routinely show background values of 1 to 1.5 ng/µL. Note that the values above will be dependent on primer concentrations, sequence, and lengths of PCR products generated.