*FluoReporter® Biotin Quantitation Assay Kit *for biotinylated nucleic acids* (F30755)*

Quick Facts

Storage upon receipt:

- ≤–20°C

Ex/Em: 495/519 nm

Introduction

The FluoReporter® Biotin Quantitation Assay Kit for biotinylated nucleic acids provides a sensitive fluorometric assay for determining the number of biotin labels on a nucleic acid. The assay is based on the displacement of a quencher dye from the biotin binding sites of Biotective™ Green reagent (Figure 1). The assay can detect from 4 to 80 pmol of biotin in a sample (Figure 2), providing a 50-fold higher sensitivity than the HABA biotin binding assay described by Green.[1]

Analysis of multiply biotinylated nucleic acids requires a preliminary nuclease digestion step to avoid underestimation caused by steric restriction of avidin binding. This kit is ideal for determining the degree of biotinylation of cDNA samples used in Affymetrix or RLS microarray protocols, and can be applied to as little as 13 ng of biotin-labeled nucleic acid (Table 1). The signal window of this assay has a Z′ factor of 0.91 (Figure 3).

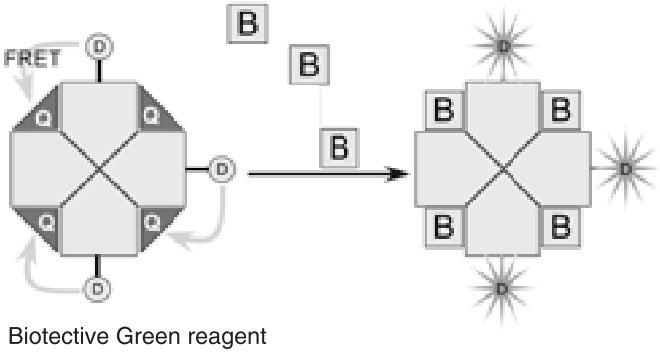

Figure 1. Schematic representation of the FluoReporter® biotin quantitation assay. The assay uses Biotective Green reagent, which consists of avidin labeled with a fluorescent dye (D) and with quencher dye ligands (Q) occupying the biotin binding sites. Through fluorescence resonance energy transfer (FRET), the ligand quenches the fluorescence. Biotin (B) attached to a nucleic acid displaces the quencher dye from Biotective Green reagent, yielding fluorescence proportional to the amount of added biotin.

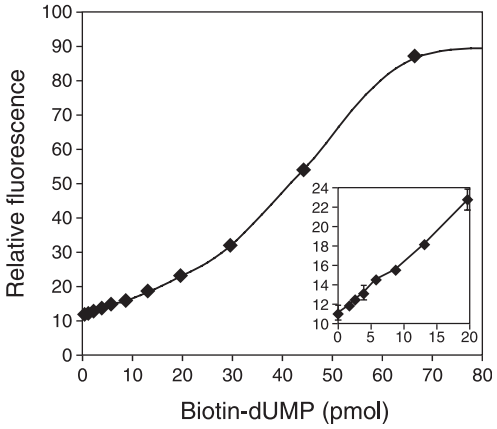

Figure 2. Standard curve showing dynamic range of the FluoReporter® Biotin Quantitation Assay Kit. Each reaction consisted of 0.5X PBS, 25 mM CHES buffer, pH 9.3, 1 mM $CaCl_2$, 1X Biotective Green reagent, and biotin-dUMP in a total volume of 100 µl. After a 5 minute incubation at room temperature in the dark, fluorescence was measured in a microplate reader using excitation at 485 ± 7.5 nm and fluorescence emission at 535 ± 12.5 nm.

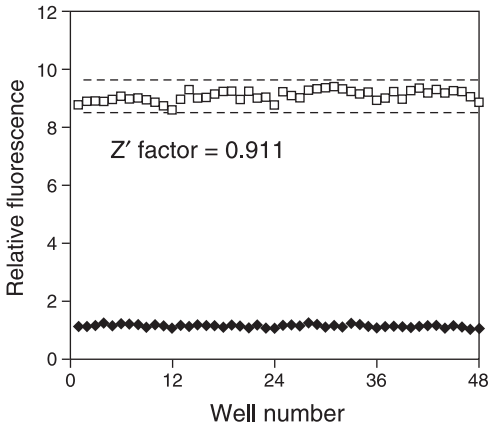

Figure 3. Z′ factor determination for the FluoReporter® Biotin Quantitation Assay Kit. The Z′ factor, a dimensionless measure of an assay's signal window,[2] provides a simple method to evaluate a high-throughput assay. For this assay, 48 wells of 0.5X PBS, 25 mM CHES buffer, pH 9.3, 1 mM $CaCl_2$, 1X Biotective Green reagent, 100 pmol biotin-dUMP in 100 µl (□) were measured alongside 48 wells of 0.5X PBS, 25 mM CHES buffer, pH 9.3, 1 mM $CaCl_2$, 1X Biotective Green reagent without biotin-dUMP (◆). The reactions were incubated and measured as described in Figure 2. The dashed lines bracketing the upper data set represent ±3 standard deviations from the mean of 48 replicates.

Table 1. Sensitivity of FluoReporter® Biotin Quantitation Assay Kit for nucleic acids with varying degrees of biotinylation.

Level of Labeling	Amount of DNA* within Range of the Assay
1 biotin: 10 bases	13.2 ng–264 ng
1 biotin: 50 bases	66 ng–1,300 ng
1 biotin: 100 bases	132 ng–2,640 ng
1 biotin: 200 bases	264 ng–5,280 ng

* To convert ng of DNA to nmol or pmol, use 330 g/mol for the molar mass of DNA.

Materials

Kit Contents

- **Biotective Green reagent** (Component A) lyophilized, 10 tubes
- **Biotin-dUMP** (Component B) 100 µl of 200 µM solution
- **Nuclease** (Component C) lyophilized, 4 vials
- **10X phosphate-buffered saline (10X PBS)** (Component D) 10 ml
- **Biotinylated DNA positive control** (Component E) (see note) 1 nmol
- **2X nucleic acid digestion buffer** (Component F) 25 ml

This kit contains sufficient reagents to assay 10 samples independently using 8 wells in triplicate for the standard curve and 3 dilutions of the sample in triplicate (totaling 33 wells per assay). However, fewer wells may be used to conserve sample, and a single standard curve can be used for multiple samples in the same experimental session.

Storage and Handling

Upon receipt, components should be stored at ≤–20°C until required for use. When stored properly, the kit components should be stable for at least 6 months. Before opening a vial, allow it to warm to room temperature.

Protocol

The following procedure is designed for use with a fluorescence multiwell plate scanner, typically in 96-well format, using a total volume of 100 µl per well. The volumes recommended here are sufficient for ~35 wells; the kit provides sufficient material for ~350 microplate wells.

Stock Solution Preparation

Allow kit components to warm to room temperature before preparing stock solutions.

1.1 Prepare 20 ml of 1X PBS by adding 2 ml of 10X PBS (Component D) to 18 ml of deionized water. This 1X PBS is sufficient for approximately 100 reactions of 100 µl each plus 10 ml excess for making stock solutions and dilutions.

1.2 Reconstitute one vial of Biotective Green reagent (Component A) with 1.75 ml of 1X PBS. This 2X reagent solution can be stored at 2–6°C for up to 5 days; DO NOT FREEZE. Each vial contains enough reagent for 33–35 reaction wells.

1.3 A positive control for the assay may be prepared by dissolving the biotinylated DNA (Component E) in 500 µl of 1X PBS (2 µM final concentration). Aliquot and store frozen.

1.4 As needed, reconstitute each vial of lyophilized nuclease (Component C) in 50 µl of 2X nucleic acid digestion buffer (Component F). This makes a 50X stock and is sufficient for treating about 50 samples (see step 2.2). For short-term use, store on ice or at 2–6°C overnight. For longer storage, aliquot and freeze at ≤–20°C until required for use. Do not vortex or subject to repeated freeze/thaw cycles.

Nuclease Digestion of Biotinylated Nucleic Acid Samples

2.1 Mix 25 µl of each biotinylated nucleic acid sample or biotinylated DNA positive control with 25 µl of 2X nucleic acid digestion buffer (Component F).

2.2 Add 1 µl of reconstituted nuclease to each 50 µl sample. Incubate for at least 60 minutes at 37°C. For >1 µg of DNA, a longer incubation (up to overnight) will be necessary.

Biotin Assay

3.1 Prepare a 1.6 µM biotin standard solution by adding 6.8 µl of 200 µM biotin-dUMP (Component B) to 843 µl of either 1X nucleic acid digestion buffer (Component F, diluted 1:1) or 1X PBS. Using the same buffer, serially dilute the 1.6 µM biotin-dUMP in triplicate columns of 50 µl per well of a 96-well microplate. Use 1X buffer without biotin-dUMP as a negative control in the final row. This dilution series will generate a standard curve of 0–80 pmol of biotin. For the most accurate quantitation, standards should be prepared in the same buffer as the experimental samples, i.e., nucleic acid digestion buffer. Standards prepared in 1X PBS will have slightly lower fluorescence than those prepared in nucleic acid digestion buffer.

3.2 Dilute the experimental samples (using the same buffer as in step 3.1) as needed based on the DNA concentration of each sample and the estimated level of biotinylation. Table 1 shows the effective quantitation range of the kit for different degrees of sample biotinylation. If you are using the positive control, dilute the digested positive control from step 2.2 to 100 nM by adding 20 µl to 180 µl of 1X nucleic acid digestion buffer.

3.3 Pipette 50 µl of the diluted samples and positive control into separate empty wells of the microplate. For the highest accuracy, we recommend that each sample be assayed in triplicate at each concentration.

3.4 Begin the reactions by adding 50 µl of Biotective Green reagent to each microplate well containing a sample or standard. Incubate for 5 minutes at room temperature, protecting the reactions from light.

3.5 Measure the resulting fluorescence in a microplate reader using typical fluorescein wavelengths (excitation/emission maxima ~485/530 nm). The signal will slowly decrease over time (~6% drop in 30 minutes). For best results, the standards and samples should be incubated with Biotective Green reagent for the same amount of time.

Data Analysis

4.1 Plot the fluorescence of the biotin standards on the x-axis against the amount of biotin on the y-axis. Do not subtract background.

4.2 Use the quadratic fit in Microsoft Excel™ software to find the quadratic equation of the standard curve from 0 to 80 pmol biotin. Insert the measured relative fluorescence of the samples as x values and solve for y (biotin amount).

4.3 An alternative to step 4.2 is to plot the standards and draw a straight line through the two points that have x values that bracket each biotinylated sample. Use the equation of this line to find the amount of biotin in each sample by inserting the measured fluorescence as the x value and solving for y.

Example: An experimental sample gave a signal of 4,000 relative fluorescence units (RFU). The standards that bracket it are 10 pmol and 20 pmol biotin, which gave 2,000 and 9,000 RFU, respectively. The equation of the line between these two standard points is y = 0.00143x + 7.143, where x is fluorescence and y is biotin amount. Solving for y, the experimental sample contains 12.9 pmol. This amount can be used to determine the concentration of the original sample.

4.4 Once you have calculated the biotin concentration of each original sample, divide that number by the concentration of the nucleic acid in the original sample. Depending on the accuracy desired, either estimate the amount of DNA in your sample, or measure the concentration exactly using our Quant-iT™ or PicoGreen® quantitation kit or the NanoDrop® spectrophotometer. The positive control has 6 moles of biotin per mole of oligonucleotide (see note).

Note

Sequence of biotinylated DNA positive control: 5′-C[BiodT]CAT AGC[BiodT]CACGC[BiodT]GTAGG[BiodT]ATC[BiodT]CAG [BiodT]TCGG-3′

References

1. Biochem J 94, 23C (1965); **2.** J Biomolecular Screening 4, 67 (1999).

Product List *Current prices may be obtained from our Web site or from our Customer Service Department.*

Cat #	Product Name	Unit Size
F30755	FluoReporter® Biotin Quantitation Assay Kit *for biotinylated nucleic acids* *10 determinations*	1 kit

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