Technical Tip: A Better Way to Quantitate the Degree of Biotinylation of Proteins

Why guess? Whether you purchase a commercially available biotinylated reagent or make your own using one of our reactive biotin derivatives, you still need to know the degree of biotinylation before you proceed with your experiment. If you're still relying on the conventional colorimetric HABA-based biotin-binding assay[1], we've got a better way.

The FluoReporter® biotin quantitation assay is based on the Biotective™ Green reagent (Figure 1). The Biotective™ Green reagent exhibits bright green fluorescence (excitation/emission maxima of 495/519 nm), making this assay compatible with standard fluorescence-based microplate readers. The FluoReporter® Biotin Quantitation Assay Kits offers several important advantages:

FluoReporter® Biotin Quantitation Assay Kit for proteins (F30751)

Detect from 4 to 80 pmol of biotin, a 50-fold higher sensitivity than the HABA-based assay. In addition, unlike the HABA-based assay, which requires ~1 mg of protein sample, the FluoReporter® Biotin Quantitation Assay Kit for proteins requires <1 µg.

FluoReporter® Biotin Quantitation Assay Kit for nucleic acid (F30755)

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 13.2 ng* of nucleic acid.

In addition to the Biotective™ Green reagent, each FluoReporter® Biotin Quantitation Assay Kit contains a biotin standard for the assay, a biotinylated positive control, protease or nuclease, and the appropriate digestions buffers. For accurate biotinylation data, rely on the FluoReporter® assay technology. Additional information on these products can be found at probes.invitrogen.com.

*Accurate results using 13.2 ng of nucleic acid containing ~1 biotin for every 10 bases or 132 ng of nucleic acid containing ~1 biotin for every 100 bases.

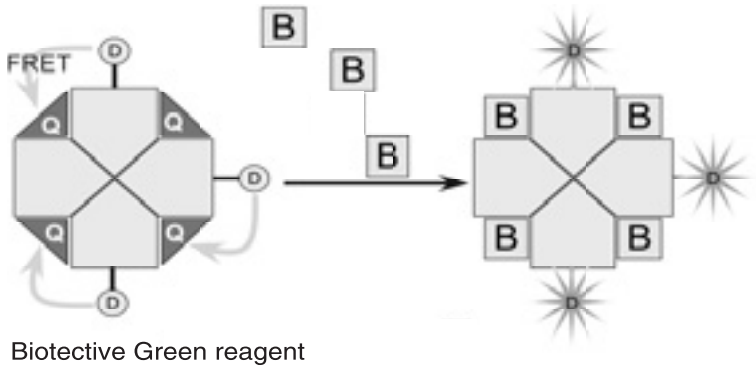

Figure 1. Schematic representation of the FluoReporter® biotin quantitation assay. This assay uses the Biotective™ Green reagent, which consists of avidin labeled with a fluorescent dye (D) and with quencher dye ligands (Q) occupying the biotin binding sites. Through fluorescence resonance energy transfer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

Reference

1. Biochem J 94, 23C (1965).

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

Cat #	Product Name	Unit Size
F30751	FluoReporter® Biotin Quantitation Assay Kit *for biotinylated proteins* *5 determinations*	1 kit
F30755	FluoReporter® Biotin Quantitation Assay Kit *for biotinylated nucleic acids* *10 determinations*	1 kit

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