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A Geno Technology, Inc. (USA) brand name

HOOK™ BiotinQuant™

(Cat. # BKC-01)

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INTRODUCTION

G-Biosciences offers a large number of biotinylation reagents for the labeling of antibodies, proteins and other molecules with biotin. The biotinylation reagents are available as reagents or in kit formats containing key labeling accessories, including HOOK™ BiotinQuant™. HOOK™ BiotinQuant™ uses HABA (2-(4-Hydroxyphenylazo)benzoic acid/ 2-(4'-Hydroxybenzeneazo)benzoic acid/ 4'-Hydroxyazobenzene-2-carboxylic acid) to rapidly estimate the mole-to-mole ratio of biotin to antibody or protein.

The method of biotin incorporation estimation is based on the binding of avidin with HABA dye, which produces a color that can be read at 500nm. The HABA-avidin complex can be displaced with free biotin or biotin conjugated with other molecules (proteins). Measuring the change in optical density of HABA-avidin complex with biotinylated proteins allows for accurate estimation of the molar ration of biotin conjugated to the protein/ antibody.

HOOK™ BiotinQuant™ is supplied with premixed, OneQuant™ HABA/Avidin aliquots to eliminate difficulties in weighing small amounts of reagents and ensuring reproducible estimations.

ITEMS SUPPLIED (Cat. # BKC-01)

Description	Size
OneQuant™ HABA/Avidin	24 vials
BiotinQuant™ Assay Buffer	25ml
Biotin Standard	1ml

STORAGE CONDITIONS

The kit is shipped at ambient temperature. Upon arrival, store at -20°C. Avoid freeze/thawing of the OneQuant™ HABA/Avidin, remove the number of vials required and keep remaining vials at -20°C.

IMPORTANT INFORMATION

- Ensure that all free/ unconjugated biotin is removed from the labeled protein or other molecule before performing an estimation. We recommend desalting with our SpinOUT™ desalting spin columns or dialysis with our micro dialysis devices, Tube-O-DIALYZER™.
- During desalting or dialysis, we recommend exchanging the reaction buffer to BiotinQuant™ Assay Buffer to ensure accurate estimation. PBS or TBS may also be used, but avoid buffers containing potassium that may result in unwanted precipitation.
- A small variation in color between the OneQuant™ HABA/Avidin does not affect the performance of the reagents.
- The Biotin Standard is supplied as an optional positive control for the assay. Use 100µl in lieu of the biotinylated sample. See calculation for determining amount of biotin in the standard.

PREPARATION BEFORE USE

1. Add 50µl ultra pure water to a vial of OneQuant™ HABA/Avidin.
2. Incubate at room temperature for 5 minutes.
3. Vortex to solubilize the HABA/Avidin.

PROTOCOL 1: CUVETTE PROTOCOL

1. Allow the reagents to warm to room temperature.
2. Pipette 850µl BiotinQuant™ Assay Buffer into a 1ml cuvette and zero the spectrophotometer at a 500nm wavelength.
3. Briefly centrifuge a OneQuant™ HABA/Avidin vial and then transfer entire contents to the cuvette and mix by gentle inversion.
4. Measure the absorbance of the HABA/Avidin complex at 500nm. This is your A_{500} HABA/Avidin reading.
5. Add 100µl biotinylated sample to the HABA/Avidin cuvette and mix well by inversion.
   NOTE: If using optional Biotin Standard, replace the 100µl biotinylated sample with 100µl Biotin Standard.
6. Measure the absorbance of the solution at 500nm. Record the absorbance once it has stabilized for 10-15 seconds. This is your A_{500} HABA/Avidin/Biotin Sample reading.
   NOTE: If the absorbance is <0.3, dilute the biotin sample and repeat the assay.
7. Go to the calculation section to determine the moles of biotin per mole of protein.

PROTOCOL 2: MICROPLATE PROTOCOL

1. Allow the reagents to warm to room temperature.
2. Pipette 170µl BiotinQuant™ Assay Buffer into each microplate well. Blank the plate reader with a well containing only BiotinQuant™ Assay Buffer.
3. Briefly centrifuge a OneQuant™ HABA/Avidin vial and then add 10µl OneQuant™ HABA/Avidin to the cuvette and mix on an orbital shaker or equivalent.
4. Measure the absorbance of the HABA/Avidin complex at 500nm. This is your A_{500} HABA/Avidin reading.
5. Add 20µl biotinylated sample to the HABA/Avidin well and mix well as before. *NOTE: If using optional Biotin Standard, replace the 20µl biotinylated sample with 20µl Biotin Standard.*
6. Measure the absorbance of the solution at 500nm. Record the absorbance once it has stabilized for 10-15 seconds. This is your A_{500} HABA/Avidin/Biotin Sample reading.
   NOTE: If the absorbance is <0.3, dilute the biotin sample and repeat the assay.
7. Go to the calculation section to determine the moles of biotin per mole of protein.

CALCULATIONS

Based on Beer Lambert (Beer's) Law: $\mathbf{A_\lambda = \varepsilon_\lambda bC}$*, where*

- **A** is the absorbance at a particular wavelength (λ). HOOK™ BiotinQuant™ assay is performed at 500nm.
- **ε** is the extinction coefficient at the wavelength (λ). For HABA/Avidin samples at 500nm, pH7.0 this is 34,000$M^{-1}cm^{-1}$.
- **b** is the path length in centimeters. Cuvettes (10x10mm) have a pathlength of 1cm. The pathlength for microplates, using the indicated volumes, is normally 0.5cm.
- **C** is the molarity concentration of the sample (= mol/L = mmol/ml)

For calculating the number of moles of biotin per mole of protein or sample the following values are required:

- Concentration of protein/sample used (mg/ml)
- Molecular weight of protein, expressed as grams per mole (e.g. IgG = 150,000)
- A_{500} HABA/Avidin reading
- A_{500} HABA/Avidin/Biotin Sample
- Dilution factor (DF), if sample was diluted before adding to HABA/avidin solution.

1. Calculate mmol biotinylated protein/ml:

$$\text{Calculation 1: } \frac{\text{protein concentration (mg/ml)}}{\text{MW of protein (mg/mmol)}} = \text{mmol protein/ml}$$

2. Calculate change in absorbance at 500nm:

Calculation 2 (Cuvette): $(0.9 \times A_{500}\ \text{HABA/Avidin}) - (A_{500}\ \text{HABA/Avidin/Biotin Sample}) = \Delta A_{500}$

Calculation 2 (Microplate): $(A_{500}\ \text{HABA/Avidin}) - (A_{500}\ \text{HABA/Avidin/Biotin Sample}) = \Delta A_{500}$

NOTE: 0.9 is the correction factor for the dilution of the HABA/Avidin with the sample in the cuvettes. This is not necessary for microplates as the dilution is offset by the increase in volume and therefore the light path (b).

3. Calculate concentration of biotin in reaction (mmol/ml):

$$\text{Calculation 3: } \frac{\Delta A_{500}}{34{,}000 \times b} = \frac{\text{Calculation 2}}{34{,}000 \times b} = \frac{\text{mmol biotin}}{\text{ml reaction mixture}}$$

NOTE: b = lightpath, which is 1cm for cuvettes and 0.5cm for microplates.

4. Calculate mmol of biotin per mmol of protein:

$$\text{Calculation 4} \quad \frac{\text{mmol biotin in original sample}}{\text{mmol protein in original sample}} = \frac{\text{mmol biotin in reaction} \times 10 \times \text{DF}}{\text{Calculation 1}} = \frac{\text{Calculation 3} \times 10 \times \text{DF}}{\text{Calculation 1}}$$

NOTE: DF is the dilution factor. 10 is for the 10 fold dilution of the biotinylated protein sample in the reaction mixture.

5. Calculate concentration of biotin in Biotin Standard (mM):

$$\text{Calculation 5: } \frac{\Delta A_{500} \times 10 \times 1000}{34{,}000 \times b} = \quad = \quad [\text{Biotin Standard}]\ (\text{mM})$$

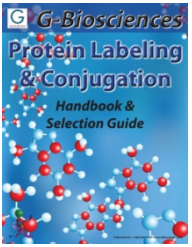
NOTE: b = lightpath, which is 1cm for cuvettes and 0.5cm for microplates. 10 is for the 10 fold dilution of the Biotin Standard in the reaction mixture.

TROUBLESHOOTING

Issue	Suggested Reason	Possible Solution
ΔA_{500} is ≤0	Low or zero biotinylation of protein.	Lack of functional groups for biotinylation, use a different coupling chemistry.
	Incomplete reagent mixing	Ensure all the OneQuant™ HABA/Avidin is fully dissolved before using
	Particulates in protein solution interfering with absorbance	Filter protein solution before assaying
	Potassium ions present in sample	Ensure samples are in BiotinQuant™ Assay Buffer
Biotin levels are unexpectedly high	Free, Unconjugated biotin not removed	Desalt or dialyze biotinylated sample before use to remove free biotin.

RELATED PRODUCTS

Download our Sample Preparation Handbook.



http://info.gbiosciences.com/complete-protein-labeling-conjugation-handbook

For other related products, visit our website at www.GBiosciences.com or contact us.

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