



HOOK™ - Biotin Kit

INTRODUCTION

Based on the protein target reactive groups, biotin reagents can be divided into amines reactive (such as N-hydroxysuccinimide (NHS) esters), sulfhydryl reactive (such as maleimide and iodoacetyl), carbohydrate reactive (such as hydrazides), and carboxyl reactive biotin reagents (such as amines). Photo-reactive biotin reagents react non-specifically upon exposure to UV light and are used when no appropriate reactive group is available on the target molecules. The HOOK™ - Biotin kit is provided with a reactive biotin reagent, ready-to-use buffers (Optimizer Buffers) for performing biotinylation reaction, Tube-O-Reactor™, and biotinylation estimation reagents. The entire labeling procedure is performed in a single Tube-O-Dialyzer™ - sample equilibration and optimization, biotin coupling reaction, and finally removal of the un-reacted biotin and storage of the reaction conjugated products for later use.

KIT COMPONENTS:

	Size
HOOK™ - Biotin Agent (Selected from the list on page 2 of the protocol)	25mg x 1* <i>*Note: HOOK™ - Psoralen-PEO-Biotin (BK-20) is supplied as only 5mg with each kit.</i>
Optimizer Buffer™ I to V [5X] (As per Optimizer Buffer™ Selection Guide)	25ml x 2
Tube-O-Reactor™ (Cat#786-024-8K)	<u>Contains:</u> 5 Tube-O-Dialyzers - <u>8kDa MWCO*</u> , <u>Micro</u> (for 20-250ul reaction volume), 5 Micro Caps, 5 Micro Floats, 5 Micro Dialysis Cups and 60 glass balls
Tube-O-Reactor™ (Cat#786-027-8K)	<u>Contains:</u> 5 Tube-O-Dialyzers - <u>8kDa MWCO*</u> , <u>Medi</u> (for 0.2-2ml reaction volume), 5 Medi Caps, 5 Medi Floats, 5 Micro Dialysis Cups and 60 glass balls
Avidin	5mg x 2
HABA Dye Reagent	1ml
BiotinQuant™ Assay Buffer	25ml

NOTE: Optimizer Buffer™ Selection Guide

Optimizer Buffer™ I [5X]	Suitable for Amine Reactive & Photoactive Reagents Supplied with Cat # BK-01 to BK-10, and BK-20
Optimizer Buffer™ II [5X]	Suitable for Sulfhydryl Reactive Reagents Supplied with Cat # BK-11 and BK-12
Optimizer Buffer™ III [5X]	Suitable for Sulfhydryl Reactive Reagents Supplied with Cat # BK-13 and BK-14
Optimizer Buffer™ IV [5X]	For Carboxyl Reactive Reagents Supplied with Cat # BK-15 to BK-17
Optimizer Buffer™ V [5X]	For Carbohydrate Reactive Reagents Supplied with Cat # BK-18 and BK-19

STORAGE CONDITION:

The kit is shipped at Ambient Temp. Upon arrival, store the kit components at 4°C, except Avidin should be stored at -20°C.

ITEMS NEEDED BUT NOT SUPPLIED WITH THE KIT:

Solvent medium for biotin agent, Shaker or Stir plate and stir bar.



HOOK™-Biotin Kits and specification of HOOK™-Biotin reagents supplied with each kit:

Catalog #	Hook-Biotin Supplied	Spacer Arm	Solvent Needed	Reaction pH	Reactive Group	Target Group	Membrane Permeability
BG-00	HOOK™-Biotin	-	-	-	-	-	-
BK-01	HOOK™ - NHS-Biotin	13.5 Å	DMF or DMSO	7-9	NHS Ester	Primary Amines	Yes
BK-02	HOOK™ - NHS-LC-Biotin	22.4 Å	DMF or DMSO	7-9	NHS Ester	Primary Amines	Yes
BK-03	HOOK™ - NHS-LC-LC-Biotin	30.5 Å	DMF or DMSO	7-9	NHS Ester	Primary Amines	Yes
BK-04	HOOK™ - NHS-SS-Biotin	24.3 Å	DMF or DMSO	7-9	NHS Ester	Primary Amines	Yes
BK-06	HOOK™ - Sulfo-NHS-Biotin	13.5 Å	Water or DMSO	7-9	Sulfo-NHS Ester	Primary Amines	No
BK-07	HOOK™ - Sulfo-NHS-LC-Biotin	22.4 Å	Water or DMSO	7-9	Sulfo-NHS Ester	Primary Amines	No
BK-08	HOOK™ - Sulfo-NHS-LC-LC-Biotin	30.5 Å	Water or DMSO	7-9	Sulfo-NHS Ester	Primary Amines	No
BK-09	HOOK™ - Sulfo-NHS-SS-Biotin	24.3 Å	Water or DMSO	7-9	Sulfo-NHS Ester	Primary Amines	No
BK-10	HOOK™ - PFP-Biotin	9.6 Å	DMF or DMSO	7-9	Pentafluorophenyl Ester	Primary or Secondary Amines	Yes
BK-11	HOOK™ - PEO-Iodoacetyl-Biotin	24.7 Å	Water or DMSO	7.5-8.5	Iodoacetyl	Sulfhydryl	No
BK-12	HOOK™ - Iodoacetyl-LC-Biotin	27.1 Å	DMF or DMSO	7.5-8.5	Iodoacetyl	Sulfhydryl	Yes
BK-13	HOOK™ - Biotin-PDA	21.1 Å	DMF or DMSO	6-9	Pyridyldithiol	Sulfhydryl	Yes
BK-14	HOOK™ - Biotin-BMMCC	35.4 Å	DMF or DMSO	6.5-7.5	Maleimide	Sulfhydryl	Yes
BK-15	HOOK™ - Biotin-Pentylamine	18.9 Å	DMF or DMSO	4-6	Amine	Carboxyl	No
BK-16	HOOK™ - Biotin-PEO-Amine	20.4 Å	Water or DMSO	4-6	Amine	Carboxyl	No
BK-17	HOOK™ - Biotin-PEO-LC-Amine	22.9 Å	Water or DMSO	4-6	Amine	Carboxyl	No
BK-18	HOOK™ - Biotin Hydrazide	15.7 Å	DMSO	4-6	Hydrazide	Carbohydrate (Oxidized)	Yes
BK-19	HOOK™ - Biotin-LC-Hydrazide	24.7 Å	DMSO	4-6	Hydrazide	Carbohydrate (Oxidized)	Yes
BK-20	HOOK™ - Psoralen-PEO-Biotin	36.9 Å	Water or DMSO	7-8	Psoralen	DNA/RNA /Protein	No

PREPARATION BEFORE USE

1. First review the product protocol for the Tube-O-Reactor™ (supplied with the kit).
2. Dilute and prepare 1X Optimizer Buffer™ (1ml 5X Optimizer Buffer™ per 4ml de-ionized water).
3. Warm the Biotin-Agent vial(s) to room temperature before opening to prevent the condensation and deterioration of the biotin agent.

SAMPLE EQUILIBRATION:

Prepare protein sample in an appropriate 1X Optimizer Buffer™ for optimal reaction (See Optimizer Buffer™ Selection Guide).

For dry sample, dissolve 2-10mg of protein in 1ml of 1X Optimizer Buffer™.

If protein solution is in an incompatible buffer, dialyze and equilibrate into 1X Optimizer Buffer™ as follows:

1. Transfer the protein sample for labeling into a Tube-O-Dialyzer™ (for 20µl to 250µl samples use Micro and for 200µl to 2.5ml samples use Medi size Tube-O-Dialyzer™).

Note: The kit is supplied with 8kDa MWCO Tube-O-Dialyzer™. Different MWCO Tube-O-Dialyzer™ (Micro or Medi size) can be ordered separately from our list (see Related Products).

2. Dialyze the sample against 3-5ml 1X Optimizer Buffer™ (or an appropriate buffer) at room temperature (or in cold depending on sample requirement) for 1-2h.

OPTIONAL: Depending on the nature of the sample, the sample may be dialyzed a second time for 1-2 h in fresh 3-5ml 1X Optimizer Buffer™ to ensure complete exchange of the buffer and equilibration of the sample.

PREPARATION OF BIOTIN AGENTS

1. Warm the biotin-agent vials to room temperature before opening.
2. Immediately before using, add appropriate solvent (200µl/ 2 mg biotin) to prepare 10 mg biotin/ml solution.

BIOTIN CONJUGATION REACTION

Note: For most cases, 20 molar excess of biotin agent to sample protein can be used to ensure sufficient biotinylation. For some proteins, the reacting molar ratio of biotin agent to protein and the reacting time may have to be adjusted. Read “*Specific Reaction Considerations*” below.

Biotin conjugation efficiency can be determined using the reagents and protocol supplied with this kit.

1. Gently loosen the Tube-O-Dialyzer™ cap and add an appropriate volume of the concentrated and freshly prepared biotin solution to give a 20 fold molar excess of biotin over protein in the reaction solution (see the calculation below).
2. Replace the cap and vortex the Tube-O-Dialyzer™ for a brief 10 seconds.
3. Invert the Tube-O-Dialyzer™ and reposition into the Micro-Dialyzer tank without any buffer and incubate the Tube-O-Reactor™ at room temperature for 30-40 minutes.

NOTE - reaction incubation conditions may be different for different biotin agents, see “*Specific Reaction Considerations*” below.

REMOVAL OF UN-CONJUGATED BIOTIN AGENTS

1. Dialyze the reaction mixture in Tube-O-Dialyzer™ against an appropriate 1X Optimizer Buffer™ (3-5ml) or any appropriate buffer of your choice for at least 2 - 4 hours and 1-2 changes of fresh dialysis buffer.
2. Store biotinylated protein at 4°C in 0.1% sodium azide until ready for use.

CALCULATION OF BIOTIN AGENT NEEDED FOR CONJUGATION

µmoles of protein = µg protein / MW protein

µmoles biotin reagent to add = µmoles protein x 20

µg biotin reagent to add = µmoles biotin to add x MW biotin*

µg biotin reagent to add can be converted to µl to add from the concentration of 10µg/µl

*MW of appropriate biotin selected

SPECIFIC REACTION CONSIDERATIONS

For sulfhydryl reactive biotin reagents (Cat. # BK-11 to BK-14):

Because these reagents only react with free sulfhydryl groups, the protein must be reduced using reducing reagents such as DTT, β-Mercaptoethanol or TECP, and free reducing reagents must be removed by dialysis against 1X Optimizer Buffer™ or the buffer of your choice before the biotinylation. For biotin reagents whose reactive group is iodoacetyl (Cat. # BK-11 and BK-12), the biotin conjugation reaction (Step 1-3) should be protected from direct light. Place the Tube-O-Reactor™ assembly in dark and cold.

For carboxyl reactive biotin reagents (Cat. # BK-15 to BK-17):

The reaction of carboxyl and biotin needs carbodiimide to mediate. Immediately before the biotin conjugation reaction, prepare 1 mg/ml EDC (*G-Biosciences* Cat. # BC25) in 1X Optimizer Buffer™ IV. Add 12ul EDC solution per ml biotin-protein reaction mixture and mix (after biotin conjugation reaction Step -1). If precipitate is formed, centrifuge for a brief 5-10 seconds at 1000xg and remove the precipitate.

For carbohydrate reactive biotin reagents (Cat. # BK-18 and BK-19):

In order to react with the hydrazide group of biotin reagent, glycoproteins must be oxidized to generate aldehyde group. Prepare 20mM sodium meta-periodate (NaIO₄) in 1X Optimizer Buffer™ V just before use. Cool the protein solution and the sodium meta-periodate solutions on ice. Add equal volume of ice-cold sodium meta-periodate solution to your protein solution and mix well. Incubate for 30 minutes on ice and in dark. Dialyze the protein solution against 1X Optimizer Buffer™ V before proceeding to the biotin conjugation reaction step.

For photoreactive biotin reagents (Cat. # BK-20):

Dissolve DNA or RNA to a concentration of 1ug/ul in 1X Optimizer Buffer™ I. Prepare 20mM HOOK™ - Psoralen-PEO-Biotin in DI-water and protect from light. Add HOOK™ - Psoralen-PEO-Biotin to the sample solution to final concentration ~200uM and mix well. Irradiate the open tube under a long wavelength UV light (~365nm) at least 10-30 minutes. After irradiation, free HOOK™ - Psoralen-PEO-Biotin can be removed by dialysis against 1X Optimizer Buffer™ I or precipitation of DNA or RNA with ethanol.

INSTRUCTIONS FOR BIOTINYLACTION OF CELL SURFACE PROTEINS

Cell Sample Preparation:

Wash and prepare cell suspension for biotin reaction.

1. Wash cells three times with ice-cold PBS buffer to remove any contaminating proteins.
2. Suspend the cells at a concentration of 25 x 10⁶ cells/ml in PBS.

Note: Other cell concentrations can be used based on cell size, type, etc. The concentration of biotinylation reagent can be scaled up or down accordingly.

Biotin Agent & Cell Surface Reaction

Prepare biotin agent as described above.

1. Add 0.5mg Biotin Agent per ml of reaction volume (solid form of biotin agent can be directly added if it is water soluble).
2. Gently mix and incubate at room temperature for 30 minutes.
3. Wash cells three times with ice-cold PBS to remove any remaining biotinylation reagent. The cells surface proteins are now biotinylated.

ESTIMATION OF BIOTIN INCORPORATION EFFICIENCY

The method of biotin incorporation estimation is based on binding of avidin with HABA dye [2-(4-hydroxyazobenzene)-benzoic acid], which produces a color that can be read at 500nm. The HABA-avidin complex can be displaced with free biotin or biotin conjugated molecules (proteins). Measuring the change in optical density of HABA-avidin complex with biotinylated proteins allows estimation of biotin conjugated with the protein.

For the estimated biotin incorporation efficiency, the kit is supplied with the following components: Avidin, HABA Dye Reagent and BiotinQuant Assay Buffer.

Preparation of Avidin-HABA Reagent:

Avidin-HABA Reagent: Dissolve 5 mg avidin in 9.6 ml BiotinQuant™ Assay Buffer and mix with 0.4 ml HABA Solution. The solution can be used for two weeks if stored at 4°C.

Protocol For Estimation of Biotin Incorporation

1. Add 0.9 ml Avidin-HABA Reagent in 1ml cuvette and measure the absorbance at 500nm (OD avidin-HABA) against Assay Buffer. Add 0.1 ml biotinylated protein sample and mix.
2. Measure the absorbance of the mix solution at 500nm when the value is stable for at least 15 seconds (OD sample-mix). If the value is not greater than 0.4, dilute the sample and repeat the assay.

Calculation of Biotin Incorporation

1. OD change = 0.9 x OD avidin-HABA - OD sample-mix
2. Concentration of bound biotin (μmoles biotin/ml) = OD change / 34
3. Concentration of sample protein (μmoles/ml)

- = μg of protein / MW protein / volume of the biotinylated protein solution (ml)
4. Moles biotin / mole protein
= (Concentration of bound biotin x dilution factor) / Concentration of sample protein

Note: 34 is the apparent absorbance change at 500nm per μmole of biotin bound per ml.

The dilution factor is 10 in this assay protocol because 0.1 ml sample mixed with 0.9ml avidin-HABA solution. Any other dilution factor used must be counted also.

RELATED PRODUCTS:

1. **Avidin (Cat. #BKC-02)** - affinity purified for estimation of biotin incorporation and other applications.

2. **HABA (Cat. #BKC-03)** - biotin estimation dye reagent.

3. **DMSO (Cat. #BKC-17) & DMF (Cat. #BKC-16)** - Vials containing un-hydrous DMSO [Dimethyl sulfoxide (CH_3)₂SO] and DMF [N,N-Dimethylformamide HCON(CH₃)₂]. Suitable for biotinylation reaction applications.

4. **Sodium meta-periodate (Cat. #BKC-15)** - Vials containing 5g sodium meta-periodate(NaIO_4). Suitable for oxidizing glycoproteins

5. **EDC (1-Ethyl-3- [3-dimethylaminopropyl] carbodiimide) (Cat. #BC25)** - Reactive group: carbodiimide, target group: amino and carboxyl groups, form amide bond. Supplied in 1g and 5g sizes.

6. **Tube-O-Array Dialyzer™ (Cat. #786-145A)** - A high throughput method for sample preparation and optimization. Specifically developed for dialysis-equilibration of samples prior to 2D-gel analysis or other applications. Optimize up to 12 samples at a time.

Note: For other related products, visit our web site at www.GBiosciences.com or contact us.

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