FT-BT3600

Chromalink Biotins

Spectrophotometrically directly quantifiable Biotinylation reagents

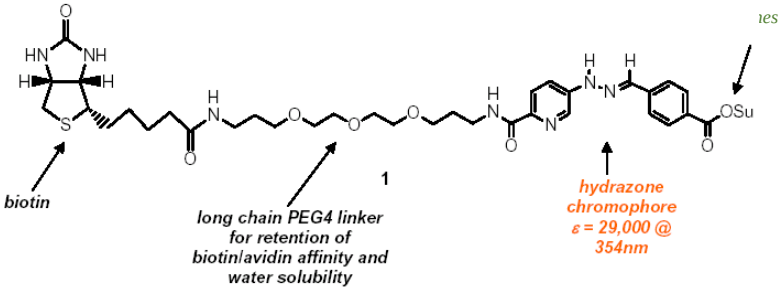
Products Description

Catalog #: BT3602, 5x1mg
BT3603, 10mg

Name: **NHS-ChromaLink™ Biotin**
Succinimidyl-PEG$_3$-Biotin 354S

Properties MW 810.92
DMF soluble

Storage: +4°C (keep under inert atm., dessicated)
(-20°C for long term) (K)

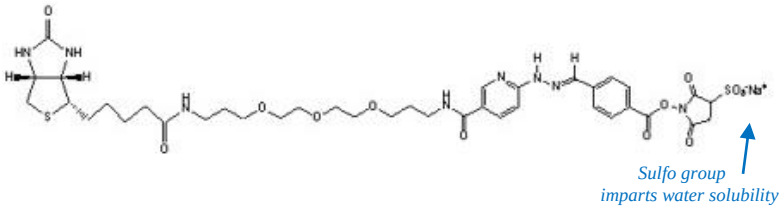


Catalog #: CE9602, 5x1mg
CE9603, 10mg

Name: **SulfoNHS-ChromaLink™ Biotin**
SulfoSuccinimidyl-PEG$_3$-Biotin 354S

Properties MW 912,9
Water soluble

Storage: -20°C (under inert atm., dessicated) (M)



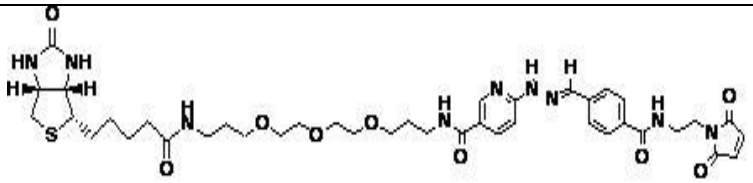
Also available as a complete labeling kit #BT3614
(contains all reagents for 5 reactions each of 25µg to 1mg of protein, with 20-200KDa)

Catalog #: CE9612, 10mg

Name: **Maleimide-ChromaLink™ Biotin**
Maleimido-PEG$_3$-Biotin 354S

Properties MW 810.92

Storage: Room temperature (under inert atm., dessicated)



Applications:
ChromaLink™ Biotin (CLB354S) NHS and Maleimide derivatives can incorporate biotin on respectively amine and sulfhydryl containing biomolecules, and allows for direct spectrophotometric quantitation of **total** biotin. ChromaLink Biotin eliminates the need to carry out cumbersome and time-consuming and poorly accurate HABA assays.
Applications include:

- Biotinylate antibodies, proteins or peptides
- Optimize biotin-avidin complex
- Quantitate the extend of biotin labeling

Benefits:

- Easy, quick and efficient biotinylation – identical protocoles as standard biotin reagents-
- Built-in signal: direct, non-destructive UV quantitation of total biotin incorporation*
- complete retention of inherent biotin/streptavidin binding
- water soluble reagent: no organic solvent needed - more convenient and safe
- PEO spacer confers water solubility and minimizing conjugate aggregation

Unique features: ChromaLink Biotin incorporates:

1-A **chromophore** that absorbs strongly at 354nm; the chromophore, once coupled to protein, is used to estimate the extend of labeling (incorporated biotin) by simple spectrophotometric measurement at two wavelengths (A280 / A354).

2-A **long polyethylene glycol (PEG3) spacer**, which confers hydrophilicity and extended length. Hence the reagent dissolves readily in aqueous buffers (no need of organic solvents), and maintains protein solubility after modification: the labeled protein is less prone to aggregation. Additionally, the extended PEG3 linker preserves biotin/streptavidin affinity, minimizing steric hindrance related to the binding of biotin-binding proteins in affinity purifications of detection applications.

3-a reactive group, that can be:

A **water soluble ester reactive group (NHS)**, known to efficiently conjugate in aqueous systems to amines, such as the ε-amino group of Lysine (K) or the α-terminus of the peptidic chain.

A **Maleimide** group that efficiently conjugate in aqueous systems at pH7-9 to sulfhydryls.

Directions for use

Below is a standard protocol to incorporate biotin into proteins for maximum. It covers a broad range of protein molecular weights (20-200KDa) and concentrations (0.25-10mg/ml), using a fixed protein volume (100µl) and either 10 or 20mole equivalents of Chromalink Biotin depending on protein samples concentration. Conditions (ratio, incubation time, temperature,...) may be optimized for special proteins, other samples (cells), or optimal labeling in a specific assay.

The protocol 1 will modify proteins with an average molar substitution ratio (MSR) of ~3-6 biotins if sufficient lysines; We do not recommend higher levels of modification since over-modified proteins often precipitate. A typical relationship between molar substitution ratio, protein concentration (mg/ml), molecular weight (Daltons), and equivalents of ChromaLink Biotin in a reaction are summarized in the technical sheet of kit #BT3614.

Protocol [1]: Protein biotinylation with ChromaLink-NHS

Biotinylation Procedure:

1) Exchange protein into 100 mM phosphate, 150 mM NaCl, pH 7.2-7.4 buffer at 2-5 mg/mL

2) Dissolve ChromaLink Biotin S354 1 (0.5 mg) in anhydrous DMF (50 µL)

3) Add an aliquot containing 10-15 mole equivalents of ChromaLink Biotin S354 1 to protein solution

4) Incubate at room temperature for 2.0 h

5) Desalt by dialysis, diafiltraton or a desalting column into desired buffer

6) Determine the molar substitution ratio (MSR: Biotin/Protein MSR) of biotin on the protein by one of two methods

Biotinylation level determination:

• Method A: Determine the A280 and A354 of the modified protein and use the following equations (NOTE: volume in mL and protein in mg/mL)

1. Determine corrected Abs280nm

$Ac_{280} = A_{280} - (A_{354} * 0.29)$ [eq. 1]

2. Determine moles of protein

[(Ac_{280} / protein 'A' value) * (volume) / 1000] / (proteinMW) = moles protein [eq. 2]

NOTE: protein 'A' value for antibodies is ~1.2

3. Determine moles of biotin present

(A_{354} / 29 000) x (volume / 1000) = moles biotin [eq. 3]

4. Biotin/protein MSR (molar substitution ratio) determination:

MSR = [eq. 3] / [eq. 2]

• Method B: Determine the protein concentration using a protein assay such as the BC Assay(UP4084) or Bradford (UPF8640) and determine the A_{354} and use the following equation:
(NOTE: volume in mL and protein in mg/mL)

1. Determine moles of protein:
   [(protein concentration) x (volume) / 1000] / protein MW= moles protein [eq. 1']
2. Determine moles biotin present (volume in mL):
   [A_{354} / 29 000) x (volume / 1000) = moles biotin [eq. 2']
3. Biotin/protein MSR (molar substitution ratio) determination:
   MSR = [eq. 2'] / [eq. 1']

• Method C: Biotin content can also be determined by the HABA method (see FT-05361D) with lower accuracy.

Protocol [2]: Protein biotinylation with ChromaLink-Maleimide

Procedure:

1. Desalt/buffer exchange the protein into Modification Buffer (100 mM sodium phosphate, 150 mM sodium chloride, pH 6.5).
This can be accomplished by any suitable method, typically be gelfiltration or by dialysis(longer but less working time, better yield, cheaper) (see related products). Notes:
a) Buffer exchange removes all small molecule contaminants, from the protein solution before modification.
b) Do not use PBS. High-level buffering capacity, i.e. 100 mM phosphate, is necessary for successful modification.

2. Determine the concentration of the protein to be modified using the BCA assay (Uptima #UP40840A). Alternatively use Bradford assay (Uptima #UPF86400) or the BCA assay (Uptima #UP40840A), or measure the A280 if the protein extinction coefficient is known (EC, ε).

3. Adjust the concentration to 1-5 mg/mL in Modification Buffer pH 7.4, if necessary.

4. Prepare a stock solution of ChromaLink Maleimido Biotin in anhydrous DMF (or DMSO) by dissolving 1-4 mg of ChromaLink in 100 µL anhydrous DMF.
Note: The ChromaLink Biotin/DMF stock solution can be stored for up to 1 week at –20°C if prepared with anhydrous DMF.

5. Add 1/10th volume of freshly prepared 10mM TCEP-HCL in molecular grade water to the buffer exchanged thiol protein.

6. Add 5-15 molar equivalents of ChromaLink Biotin stock solution to the protein solution.
Note: ref to protocol1 or use the ChromaLink Biotin Modification Calculator.

7. Allow reaction to incubate at room temperature for 1 hour.

8. Desalt/buffer exchange the biotinylated protein into your buffer of choice as directed in part A.

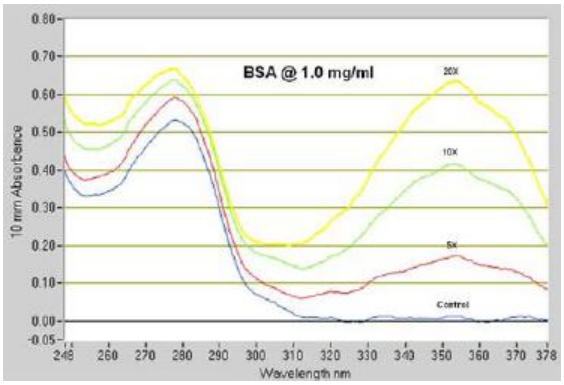
9. Quantify protein and biotin molar substitution ratio –optionnal–
1. Take a UV spectra of the biotin labeled protein. Record the A280 and A354.
Alternatively determine the concentration of the biotin labeled protein as in point 2 (BCA).
The biotin incorporation (molar substitution ratio (MSR)) can be determined using the Biotin MSR Calculator by plugging in the absorbance peaks at A280nm [or protein concentration (A354nm/Bradford or BCA Method)] and A354 (A280/354 Method). For optimal labeling, the biotin MSR should be between 1 and 4, depending on the available thiols on the protein.

The protein is now biotin labeled and ready for conjugation to streptavidin coated molecules or surfaces or other uses.

Technical and Scientific Information

- Chromalink technology

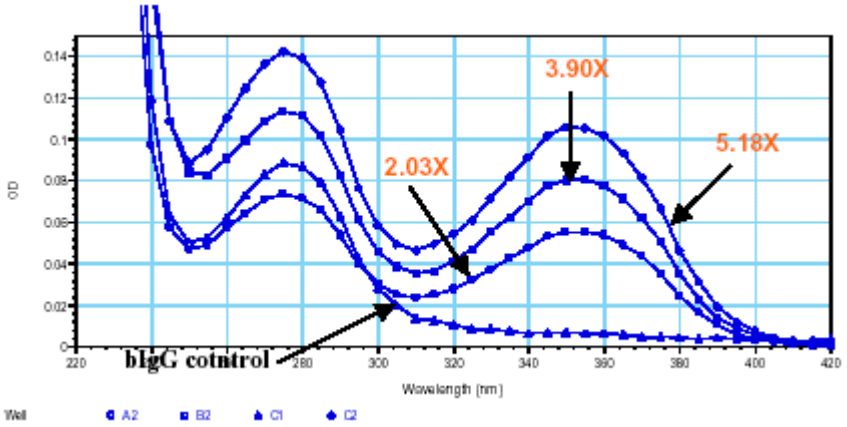
ChromaLink spacer (CLB354S) has been designed to a/ incorporate a bis-aryl hydrazone chromophore (ε 29,000 @ 354 nm) in the chain that allows for direct spectroscopic quantitation of total incorporated biotins, b/ a long chain PEG3 linker to preserve biotin/avidin affinity as well as increase solubility and c/ an aromatic succinimidyl ester that more efficiently modifies amines in aqueous buffers than aliphatic succinimidyl esters.
By measuring the A280 and A354 of the modified biomolecule the protein concentration and number of biotins incorporated/protein can be determined directly. As little as 20 µg aliquot of modified protein in 100 µL buffer in a microplate assay will yield reproducible results.



Example Application1:
Superimposed spectra of BSA biotinylated using ChromaLink Biotin. Various biotin-to-protein mole equivalents (5X, 10X and 20X) were used. Note the UV-signature at 354nm indicating incorporation of biotin. All spectra were scanned on a Molecular Dynamics SpectraMax PlusTM UV-VIS plate reader (220-420 nm).

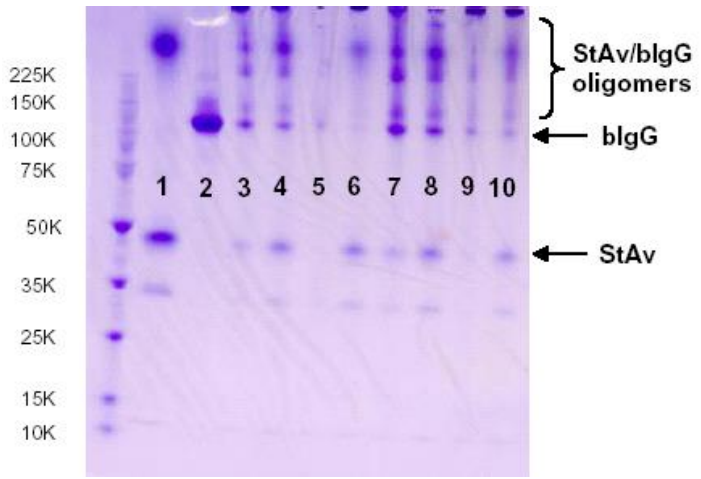
Example Application2:
The following procedure was used to modify bovine IgG (bIgG) with ChromaLink Biotin-NHS.
Bovine immunoglobulin(bIgG) was dissolved in modification buffer (100 mM phosphate, 150 mM NaCl, pH 7.2) to prepare a 5 mg/mL solution. A solution of ChromaLink Biotin NHS (0.5 mg) dissolved in DMF (50 µL) was prepared. Three separate reactions were performed wherein 5 mol equiv, 10 mol equiv and 15 mol equiv of 1 (1.3, 2.6 and 3.9 µL) respectively, were added to 0.5 mg bIgG solution. The reaction was allowed to incubate at room temperature for 2 hours. The reaction mixtures were desalted into PBS using Biomax diafiltration apparatus (Millipore).

Protein concentrations of all the modified proteins were determined using the BCA assay. Spectral analyses of each product were performed by diluting 20 µg of modified protein to 100 µL in PBS. The number of moles of chromophore incorporated was calculated by determining the absorbance of the protein at A354nm dividing by the molar extinction coefficient, i.e. 29 000, of the chromophore. The overlaid spectra of the products (0.2 µg/µL) as well as unmodified bIgG are presented below.
The number of incorporated biotins was also analyzed by the HABA assay.



	biotin/bIgG HABA	*biotin/bIgG A354*
5X	1.03	2.03
10X	1.60	3.90
15X	2.22	5.18

To both demonstrate the retention of streptavidin-binding efficiency and to compare ChromaLink Biotin to biotin-PEG$_4$-NHS, bIgG was labeled with 5X and 10X ChromaLink Biotin and biotin-PEG$_4$-NHS. The two sets of biotin-modified bIgG were treated with 1 and 2 mole equivalents of streptavidin and the binding was determined by PAGE. The PAGE gel and description of the results are presented below:



		StAv added
1	streptavidin	
2	bIgG	
3	bIgG-(CL biotin)2.03X	1.0
4	bIgG-(CL biotin)2.03X	2.0
5	bIgG-(CL biotin)3.90X	1.0
6	bIgG-(CL biotin)3.90X	2.0
7	bIgG-(PEG4/ biotin)2.03X	1.0
8	bIgG-(PEG4/ biotin)2.03X	2.0
9	bIgG-(PEG4/ biotin)3.90X	1.0
10	bIgG-(PEG4/ biotin)3.90X	2.0

Example Application2b: bIgG was reacted with 5X and 10X ChromaLink Biotin 354S and 5X and 10X biotin/PEG4/succinimidyl ester at 5mg/mL and desalted into PBS (see above). The protein concentration was determined using the BCA assay and the level of modification was determined spectrophotometrically (extinction coefficient 29000 at 354 nm). The modified proteins were reacted with 1X and 2X streptavidin and the binding determined by PAGE gel (coomassie blue stain).

The results demonstrate increasing streptavidin binding efficiency with increasing biotin modification. The lack of complete binding at the 2.4X modification level is ascribed to inaccessibility of some biotins as they may be 'hiding' in hydrophobic pockets. The results also demonstrate that ChromaLink Biotin 354S binds to streptavidin identically to bIgG modified with biotin/PEG4/succinimidyl ester.

Troubleshooting

Problem	Possible Cause	Solution
Protein was not biotin labeled or poorly labeled.	Protein was not sufficiently reduced using TCEP	React the Protein with TCEP at the same time as the reaction with the Chromalink Maleimide Biotin
ChromaLink Maleimide Biotin was hydrolyzed	Wet or poor quality DMF/DMSO hydrolyzed the maleimide group	Do not store the ChromaLink Maleimide Biotin for more than 1 week in wet DMF solvents
Molar substitution readings are out of detectable range	Protein concentrations are out of recommended range	Concentrate or dilute protein samples into recommend range
Precipitation of protein on modification	Precipitation of biotin modified proteins may occur due to a drastic change in the isoelectric properties of the modified protein.	After the biotinylation reaction is complete, addition of 1M Tris (pH 9.0) can sometimes be used to resuspend the biotinylated protein by adjusting the pH above the pI of the protein

Other Information

Related products

-Desalting tools: CelluSep dialysis , desalting columns …)

-Protein assays: BCAssay #UP4084A, CooAssay (Bradford) #UPF86400

-Other Biotinylation reagents

- Maleimido-Biotins (FT-05361D); IminoBiotin reagents (FT-39375A)

-Other crosslinkers () and other conjugation technologies:

- SMCC #UP17412, SMCC-hydrazide #BI1281,
- Hydrazone chemistry: Conjugation kit #BL1501 and crosslinkers (amine reactive SANH #BL9270, SH reactive MHPH #BL9401)

-Useful modifiers ()

- SATA #84235A, Iminothiolane #42425A
- PEGylation reagents (linkers, crosslinkers and labels) FT-DZ3531

-See Products Highlights, BioSciences Innovations catalogue and e-search tool.

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