Protein Analysis and Modification 2

protein chemistry kits at-a-glance*

Protein Tools	Absorption	Fluorescence	Other
Biotin Quantitation	**5522**		
Biotinylation			**5521**
BSA Conjugation			**5501**
KLH Conjugation			**5502**
Maleimide Quatitation		**5523**	
Protein Quantitation		**11100**	
Thiol Quantitation		**5524**	

* Products listed by catalog number

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Amplite™ Colorimetric Biotin Quantitation Kit

Cat #	Size	Storage Condition
5522	1 kit	< - 15 °C

The avidin/streptavidin-biotin interaction is the strongest known non-covalent biological interaction ($K_d = 10^{-15} M^{-1}$) between a protein and its ligand. The bond formation between biotin and avidin/streptavidin is very rapid and unaffected by pH, organic solvents, and other denaturing agents. Both avidin and streptavidin have essentially irreversible biotin-binding properties since bound biotin can only be released by denaturing the subunits of the proteins. The tight and specific binding of biotin and its derivatives to various avidins has been extensively explored for a number of biological applications.

Amplite™ Colorimetric Biotin Quantitation Kit is used for determining the molar ratio of biotin incorporated into a protein using the HABA-Avidin method. The HABA dye (4´-hydroxyazobenzene-2-carboxylic acid) binds to avidin to produce a yellow-orange colored complex which absorbs at 500 nm. Free biotin will displace the HABA dye and cause the absorbance to decrease. A standard curve can be established using the free biotin to estimate the number of moles of biotin incorporated after biotinylating a protein. The kit provides a convenient method for estimating the molar ratio of biotin to protein in biotin-protein conjugates or for quantitating biotin concentration in a solution. It is best used to determine biotin concentration in the range from 1 to 20 µM.

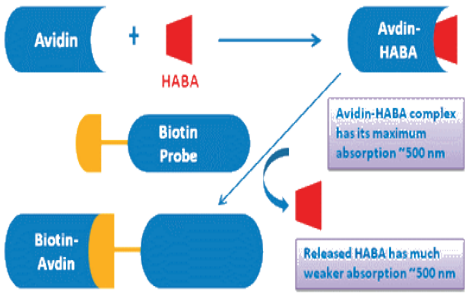


Figure 2.1. HABA Assay principle for quantifying biotinylation degree.

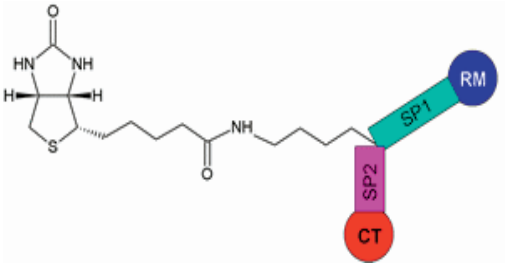
ReadiLink™ Protein Biotinylation Kit
* Powered by ReadiView™ Biotin Visionization Technology*

Cat #	Size	Storage Condition
5521	1 kit	< - 15 °C

Biotin is widely used for labeling biomolecules, in particular, antibodies. This kit is primarily optimized for the preparation of biotin-labeled antibodies for enzyme immunoassay. However, it can also be used to label any proteins and biomolecules that contain primary amines. The kit uses our ReadiView™ Biotin (3059) that reacts with an amino group of antibodies and other biomolecules. Our unique biotin contained in the kit carries a color tag to indicate the degree of biotinylation, thus eliminating the troublesome HABA biotinylation determination step. The HABA biotinylation assay is notoriously inaccurate although many efforts have been taken to improve the HABA assay accuracy (including our kit 5522). The color tag is carefully selected to avoid the interference of either biotin binding or fluorescence detection.

This kit contains all the necessary reagents for labeling and purification. On our hands, 5 to 8 biotin molecules can be conjugated to each IgG molecule using this kit. The kit is designed for 3 conjugation reactions. For each conjugation reaction the amount of material can label up to 10 mg protein. The entire process only takes less than an hour. The degree of biotinylation can be readily calculated by the following equation with a simple absorption spectrum.

$$\text{Number of Biotin/Conjugate} = [A_{351}/29300] \div [A_{280}/\xi_{protein}]$$



CT=Color Tag, RM=Reactive Moiety, SP=Spacer

Figure 2.2. The chemical structure of ReadiView™ biotin.

Related Products

Cat #	Size	Product Name
3002	100 mg	Biotin, succinimidyl ester
3010	25 mg	Biotin-X, succinimidyl ester
3050	25 mg	ReadiView™ Biotin acid
3053	5 mg	ReadiView™ Biotin amine
3055	5 g	ReadiView™ Biotin hydrazide
3058	5 mg	ReadiView™ Biotin maleimide
3059	5 mg	ReadiView™ Biotin succinimidyl ester

ReadiLink™ BSA Conjugation Kit *For Antibody Development*

Cat #	Size	Storage Condition
5501	1 kit	< - 15 °C

Bovine serum albumin (BSA) is the most abundant protein used for numerous biochemical applications including ELISAs (Enzyme-Linked Immunosorbent Assays), immunoblots, and immunohistochemistry. Like most abundant plasma proteins, BSA is very stable and soluble. In addition, the 67 kDa protein is sufficiently large and complex to be fully immunogenic. It contains numerous sites per molecule for effective conjugation of peptides and other antigens using amine-reactive or carboxy-reactive crosslinkers. Consequently, BSA is a popular carrier protein for conjugation to haptens and other weak antigens to make them more immunogenic for antibody production. This ReadiLink™ BSA Conjugation kit is primarily optimized for the simple preparation of hapten-carrier conjugates for immunization and antibody production.

Our ReadiLink™ BSA Conjugation Kit provides a one-step conjugation method of a hapten to a carrier protein using the carboxy-reactive carbodiimide as the crosslinker. The resulting conjugate is used for eliciting an immune response and antibody production against the hapten. The carboxy-reactive carbodiimide reacts with exposed carboxy and amino groups on peptides and proteins to form stable bonds. The kit contains BSA formulated in buffers compatible with the carboxy-reactive carbodiimide reactions and desalting spin columns, which offer exceptional protein recovery with a simple centrifugation step.

ReadiLink™ KLH Conjugation Kit *For Antibody Development*

Cat #	Size	Storage Condition
5502	1 kit	2-6 °C

Keyhole Limpet Hemocyanin (KLH) is one of the most commonly used carrier proteins in the conjugation of peptides for antibody production. Mariculture keyhole limpet hemocyanin (mcKLH) is a hemocyanin from the *Concholepas concholepas* mollusk with immunogenic properties similar to those of KLH. However, mcKLH is a more stable and efficient carrier protein for the production of antibodies to haptens and peptides. It contains numerous sites per molecule for effective conjugation of peptides and other antigens using amine-reactive or carboxy-reactive crosslinkers. mcKLH is currently the industry standard for antibody production against a hapten or peptide. This ReadiLink™ KLH Conjugation Kit is primarily optimized for the simple preparation of hapten-carrier conjugates for immunization and antibody production.

Our ReadiLink™ KLH Conjugation Kit provides a one-step conjugation method of a hapten to a carrier protein using the carboxy-reactive carbodiimide as the crosslinker. The resulting conjugate is used for eliciting an immune response and antibody production against the hapten. The carboxy-reactive carbodiimide reacts with exposed carboxy and amino groups on peptides and proteins to form stable bonds. The kit contains mcKLH formulated in buffers compatible with the carboxy-reactive carbodiimide reactions and desalting spin columns, which offer exceptional protein recovery with a simple centrifugation step.

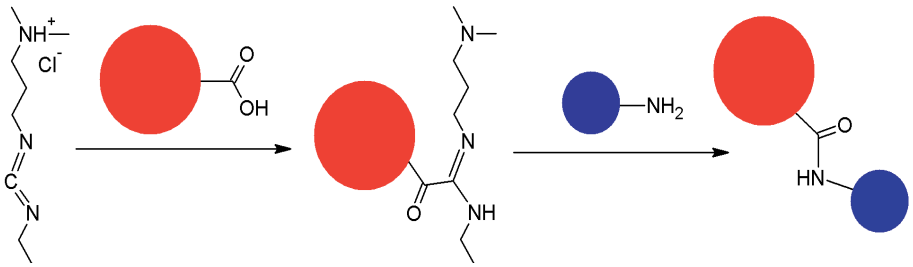


Figure 2.3. EDC reacts with a carboxyl group of carrier protein BSA or KLH (represented by the red ball), forming an amine-reactive *O*-acylisourea intermediate (the central molecule). The *O*-acylisourea intermediate reacts with an amine group on the antigen molecule represented by the smaller blue ball, yielding a conjugate of the two molecules joined by a stable amide bond [Please note the *O*-acylisourea intermediate is also susceptible to hydrolysis, making it unstable and short-lived in aqueous solution].

Related Products

Cat #	Size	Product Name
5522	1 kit	Amplite™ Colorimetric Biotin Quantitation Kit
4507	100 mg	3-Maleimidopropionic acid N-hydroxysuccinimide ester
5521	1 kit	ReadiLink™ Protein Biotinylation Kit *Powered by ReadiView™ Biotin Visionization Technology*
4501	25 mg	SMCC [4-(N-Maleimidomethyl)cyclohexanecarboxylic acid N-hydroxysuccinimide ester]
4502	1 g	SMCC [4-(N-Maleimidomethyl)cyclohexanecarboxylic acid N-hydroxysuccinimide ester]
4503	5 mg	SMCC Plus™ *Enhanced water solubility, crosslinking efficiency and stability*
4505	25 mg	Sulfo-SMCC [4-(N-Maleimidomethyl)cyclohexane-1-carboxylic acid 3-sulfo-N-hydroxysuccinimide ester, sodium salt]

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Amplite™ Colorimetric Maleimide Quantitation Kit

Cat #	Size	Storage Condition
5525	1 kit	< - 15 °C

Maleimides can be directly assayed spectrophotometrically at 302 nm. However, the small extinction coefficient of 620 $M^{-1}cm^{-1}$ renders this assay insensitive, and the assay is further complicated by the protein absorbance at the same wavelength.

This colorimetric maleimide assay kit quantifies maleimide groups by first reacting a sample with a known amount of thiol present in excess and then assaying the remaining unreacted thiol using 4,4'-DTDP with a molar extinction coefficient of 19,800 $M^{-1}cm^{-1}$. The amount of maleimide is calculated as the difference between the initial amount of thiol and the amount of unreacted thiol after the complete reaction of all maleimide groups. This spectrophotometric assay for the determination of maleimide groups is a reverse GSH assay. It takes advantage of the high reactivity of thiols of GSH with the maleimide moiety. Maleimide of the sample is allowed to form a stable thiosuccinimidyl linkage with GSH. After the reaction of the sample is complete, the excess GSH, i.e., the remaining thiols of GSH in the reaction mixture, is estimated by using 4,4'-DTDP. The amount of GSH reacted with the sample is titrated to determine the extent of maleimide.

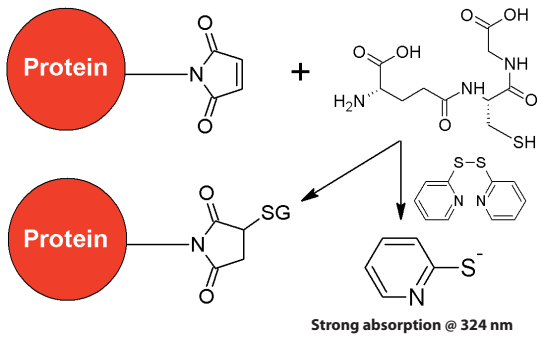

Figure 2.4. 4,4'-DTDP Assay principle for quantifying Malemide.

Amplite™ Fluorimetric Maleimide Quantitation Kit *Green Fluorescence*

Cat #	Size	Storage Condition
5523	1 kit	< - 15 °C

Sensitive assays of maleimide and thiol groups are required for the efficient conjugation of proteins that are expensive and available only in small amounts. A variety of crosslinking reagents with a maleimide group are widely used for crosslinking proteins to proteins or proteins to other biomolecules. There are few reagents or assay kits available for quantifying the number of maleimide groups that are introduced into the first protein. All the commercial kits have tedious protocols.

Our Amplite™ Fluorimetric Maleimide Qutitation kit uses a proprietary dye that has enhanced fluorescence upon reacting with a maleimide. The kit provides a sensitive, one-step fluorimetric method to detect as little as 10 picomoles of maleimide in a 100 µL assay volume (100 nM). The assay can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation without a separation step. Its signal can be easily read by a fluorescence microplate reader at Ex/Em = 490/520 nm. Compared to kit 5525, this fluorometric assay is more sensitive, and has less interference from biological samples.

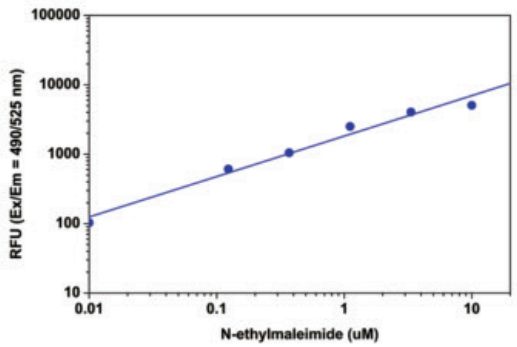

Figure 2.5. N-ethylmaleimide dose response was measured in a 96-well black solid plate with Amplite™ Fluorimetric Maleimide Quantitation Assay Kit using a NOVOStar microplate reader (BMG Labtech). As low as 0.1 µM (10 picomol/well) of maleimide can be detected with 10 minutes incubation time (n=3).

Related Products

Cat #	Size	Product Name
634	5 mg	*b*BBr [Dibromobimane] *UltraPure Grade*
633	25 mg	*m*BBr [Monobromobimane] *UltraPure Grade*
635	10 mg	*m*BCl [Monochlorobimane] *UltraPure Grade*
820	25 mg	Fluorescamine *UltraPure Grade*
825	25 mg	NBD-Cl [4-Chloro-7-nitrobenzofurazan] *UltraPure grade*
821	5 mg	NBD-F [4-Fluoro-7-nitrobenzofurazan] *UltraPure grade*
21508	5 mg	Thiolite™ Green

Amplite™ Fluorimetric Fluorescamine Protein Quantitation Kit *Blue Fluorescence*

Cat #	Size	Storage Condition
11100	1 kit	< - 15 °C

Protein quantification is necessary in protein purification, electrophoresis, cell biology, molecular biology, and other research applications. Biuret, Lowry, BCA and Bradford assays are routinely used for estimating protein concentration. However, these colorimetric assays are less sensitive, and require large sample volume to ensure higher accuracy. Our fluorescamine-based protein quantification kit is significantly more sensitive than existing standard colorimetric measurements, e.g., Bradford and Bicinchoninic acid (BCA) assays.

Fluorescamine is intrinsically nonfluorescent but reacts rapidly with primary aliphatic amines, including those in peptides and proteins, to yield a blue-green-fluorescent derivative. The Amplite™ Fluorescamine Protein Quantitation Kit provides a simple method for quantifying protein concentration in solutions. As little as 3 µg/mL of BSA can be detected. The kit can be performed in a convenient 96-well or 384-well microtiter-plate format. It can be completed within 30 minutes with the fluorescence signal easily monitored at Ex/Em = 380/470 nm. This kit has been used for (1). studying protein/protein interactions; (2). measuring column fractions after affinity chromatography; (3). estimating percent recovery of membrane proteins from cell extract; and (4). high-throughput screening of fusion protein.

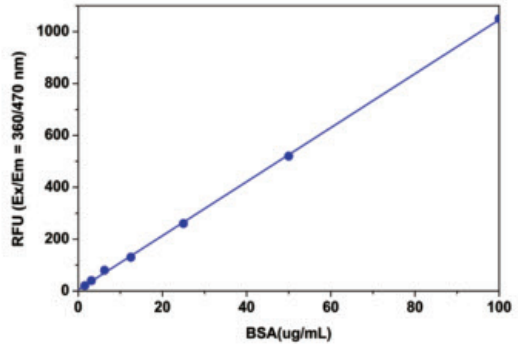

Figure 2.6. BSA dose response was measured on a 96-well back solid plate with the Amplite™ Fluoremetric Fluorescamine Protein Quantitation Kit. As low as 3 µg/mL of BSA can be detected with 5 minutes incubation time (n=3).

Amplite™ Fluorimetric Thiol Quantitation Assay Kit *Green Fluorescence*

Cat #	Size	Storage Condition
5524	1 kit	< - 15 °C

The detection and measurement of free thiol (such as free cysteine, glutathione, and cysteine residues in proteins) is one of the essential tasks for investigating biological processes and events in many biological systems. There are a few reagents or assay kits available for quantitating thiol content in biological systems. All the commercial kits either lack sensitivity or have tedious protocols.

Our Amplite™ Fluorimetric Thiol Qutitation Assay Kit provides an ultrasensitive fluorimetric assay to quantitate thiol content that exists either in a small molecule or on a protein. The proprietary non-fluorescent dye used in the kit becomes strongly fluorescent upon reacting with thiol. The kit can detect as little as 1 picomole of cysteine or GSH in a 100 µL assay volume (10 nM). The assay can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation without a separation step. The thiol sensor used in the kit generates a strongly fluorescent adduct upon reacting with a thiol compound. The resulted adduct has the spectral proterties almost identical to those of fluorescein. In addition, both absorption and emission spectra of the thiol adduct are pH-independent, making this assay kit highly robust. The signal can be easily read by a fluorescence microplate reader at Ex/Em = 490/520 nm.

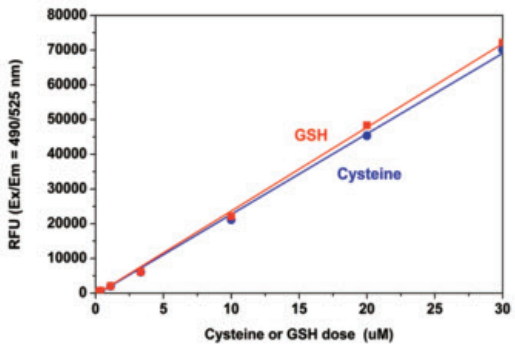

Figure 2.7. GSH and cysteine dose response was measured on a 96-well black solid plate with Amplite™ Fluorimetric Thiol Quantitation Assay Kit using a NOVOstar microplate reader (BMG Labtech). As low as 10 nM (1 pmol/well) of GSH or Cysteine can be detected with 10 minutes incubation time (n=3).

Related Products

Cat #	Size	Product Name
820	25 mg	Fluorescamine *UltraPure Grade*
634	5 mg	*b*BBr [Dibromobimane] *UltraPure Grade*
633	25 mg	*m*BBr [Monobromobimane] *UltraPure Grade*
635	10 mg	*m*BCl [Monochlorobimane] *UltraPure Grade*
821	5 mg	NBD-F [4-Fluoro-7-nitrobenzofurazan] *UltraPure Grade*
825	25 mg	NBD-Cl [4-Chloro-7-nitrobenzofurazan] *UltraPure Grade*
21508	5 mg	Thiolite™ Green

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