

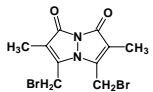
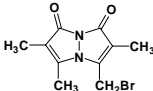
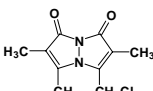
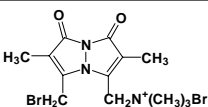
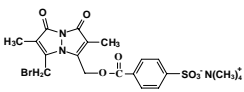
## PROTEIN MODIFICATION TOOLS

### THIOLYTE<sup>®</sup> Labeling Reagents, Biotinylating Reagents, and Crosslinkers

#### THIOLYTE<sup>®</sup> Fluorescent Labeling Reagents

THIOLYTE<sup>®</sup> Reagents are essentially nonfluorescent compounds which are capable of reacting with thiol groups to yield highly fluorescent products. THIOLYTE<sup>®</sup> Reagents form chemically stable, covalent bonds under physiological conditions, producing photolytically stable products that are easily detectable even at the picomole level. They provide an excellent alternative to other well known fluorescent labeling reagents such as fluorescein and rhodamine isothiocyanates, which yield slower reactions and generate products that fade relatively fast during irradiation.

#### Properties of THIOLYTE<sup>®</sup> Reagents

Reagent	Cat. No.	Structure	Excitation/ Emission $\lambda_{\max}$ (nm)	Comments	Refs.
THIOLYTE <sup>®</sup> DB (Dibromobimane)	596102-Y		385/477	Membrane permeant, bifunctional reagent for intra- and intermolecular crosslinking of proteins. Useful for labeling proteins in intact cells, in cell lysates, and in purified preparations.	1,2
THIOLYTE <sup>®</sup> MB (Monobromobimane)	596105-Y		385/484	Membrane permeant, monofunctional reagent; most frequently used product of the series. Labels proteins and low molecular weight non-protein thiols in intact cells, in cell lysates, and in purified preparations.	3,4
THIOLYTE <sup>®</sup> MC (Monochlorobimane)	475906-Y		380/461	Membrane permeant, monofunctional probe of choice for flow cytometric measurement of reduced glutathione (GSH) in cells. Derivatization of GSH is catalyzed by glutathione-S-transferase.	5,6
THIOLYTE <sup>®</sup> MQ (Monobromotri- methylammonio- bimane)	596106-Y		370/480	Membrane impermeant, water soluble, monofunctional quaternary salt. Labels protein thiols extracellularly, in cell lysates, and in purified preparations.	7
THIOLYTE <sup>®</sup> SB ( <i>p</i> -Sulfobenzoyl- oxybromobimane)	596110-Y		385/484	Membrane impermeant, water soluble, monofunctional anionic reagent. Yields more stable derivatives than MQ. Exhibits reactivity and sensitivity similar to MB, but its derivatives of polycationic thiols produce sharper peaks by HPLC.	8

#### REFERENCES:

- Bonet, A., et al. 1988. *Biochem. Biophys. Res. Commun.* **152**, 1.
- Kim, J.S., and Raines, R.T. 1995. *Anal. Biochem.* **225**, 174.
- Mansoor, M.A., et al. 1992. *Anal. Biochem.* **200**, 218.
- Zangerle, L., et al. 1992. *J. Neurochem.* **59**, 181.
- Hedley, C.W., and Chow, S. 1994. *Cytometry* **15**, 349.
- Young, P.R., et al. 1994. *Biochim. Biophys. Acta* **1201**, 461.
- Fahey, R.C., et al. 1980. *Anal. Biochem.* **107**, 1.
- Newton, G.L., et al. 1992. *Anal. Biochem.* **201**, 30.

## Crosslinkers

Chemical crosslinkers are used to covalently modify proteins for studying ligand-receptor interactions, conformational changes in tertiary structure, or for protein labeling. Crosslinkers are divided into homobifunctional crosslinkers, containing two identical reactive groups, or heterobifunctional crosslinkers, with two different reactive groups. Heterobifunctional crosslinkers allow sequential conjugations, minimizing polymerization.

### Homobifunctional

Reagent	Cat. No.	Modified Group	Solubility	Comments	Refs.
BMME	442635-Y	-SH	DMF, Acetone	Homobifunctional crosslinker useful for formation of conjugates via thiol groups.	9
BSOCOES	203851-Y	-NH <sub>2</sub>	Water	Base cleavable crosslinker useful for studying receptors and mapping surface polypeptide antigens on lymphocytes.	10
DSP	322133-Y	-NH <sub>2</sub>	Water	Thiol cleavable crosslinker used to immobilize proteins on supports containing amino groups.	11
DSS	322131-Y	-NH <sub>2</sub>	Water	Non-cleavable, membrane impermeable crosslinker widely used for conjugating radiolabeled ligands to cell surface receptors and for detecting conformational changes in membrane proteins.	12
EGS	324550-Y	-NH <sub>2</sub>	DMSO	Hydroxylamine cleavable reagent for crosslinking and reversible immobilization of proteins through their primary amine groups. Useful for studying structure-function relationships.	13,14
EGS, Water Soluble	324551-Y	-NH <sub>2</sub>	Water	Water soluble version of EGS that reacts rapidly with dilute proteins at neutral pH. Crosslinked proteins are readily cleaved with hydroxylamine at pH 8.5 for 3-6 hours, 37°C.	15
Glutaraldehyde	354400-Y	-OH	Water	Used for crosslinking proteins and polyhydroxy materials. Conjugates haptens to carrier proteins; also used as a tissue fixative.	16
SATA	573100-Y	-NH <sub>2</sub>	DMSO	Introduces protected thiols via primary amines. When treated with hydroxylamine, yields a free sulhydryl group that can be conjugated to maleimide-modified proteins.	17

### Heterobifunctional

Reagent	Cat. No.	Modified Group	Solubility	Comments	Refs.
GMBS	442630-Y	-NH <sub>2</sub> , -SH	DMSO	Heterobifunctional crosslinker useful for preparing enzyme-antibody conjugates (e.g. $\beta$ -gal-IgG) and for immobilizing enzymes on solid supports.	18,19
MBS	442625-Y 442626-Y	-NH <sub>2</sub> , -SH -NH <sub>2</sub> , -SH	DMSO Water	Thiol cleavable, heterobifunctional reagent especially useful for preparing peptide-carrier conjugates and conjugating toxins to antibodies.	20
PMPI	528250-Y	-SH <sub>2</sub> , -OH	DMSO, DMF	Used in the preparation of alkaline phosphatase conjugates of estradiol, progesterone, serine-enriched peptides, and vitamin B <sub>12</sub> .	21
SMCC	573114-Y 573115-Y	-NH <sub>2</sub> , -SH -NH <sub>2</sub> , -SH	DMF, AN Acetonitrile Water	Heterobifunctional reagent for enzyme labeling of antibodies and antibody fragments. The cyclohexane bridge provides extra stability to the maleimide group. Ideal reagent for preserving enzyme activity and antibody specificity after coupling.	22
SPDP	573112-Y	-NH <sub>2</sub> , -SH	DMF, AN Acetonitrile	Introduces protected thiol groups to amine groups. Thiolated proteins can be coupled to a second molecule via an iodoacetamide or maleimide group, or to a second pyridyldisulfide containing molecule.	23

#### REFERENCES:

- Weston, P.D., et al. 1980. *Biochem. Biophys. Acta.* **612**, 40.
- Howard, A.D., et al. 1985. *J. Biol. Chem.* **260**, 10833.
- Lee, W.T., and Conrad, D.H. 1985. *J. Immunol.* **134**, 518.
- D'Souza, S.E., et al. 1988. *J. Biol. Chem.* **263**, 3943.
- Geisler, N., et al. 1992. *Eur. J. Biochem.* **206**, 841.
- Moenner, M., et al. 1986. *Proc. Natl. Acad. Sci. USA* **83**, 5024.
- Yanagi, T., et al. 1989. *Agric. Biol. Chem.* **53**, 525.
- Harlow, E., and Lane, D. 1988. *Antibodies: A Laboratory Manual*, Cold Spring Harbor Publications, N. Y., p. 349.
- Duncan, R.J.S., et al. 1983. *Anal. Biochem.* **132**, 68.
- Kitagawa, T., et al. 1983. *J. Biochem.* **94**, 1160.
- Rusin, K.M., et al. 1992. *Biosens. Bioelectron.* **7**, 367.
- Green, N., et al. 1982. *Cell* **28**, 477.
- Aithal, H.N., et al. 1988. *J. Immunol. Methods* **112**, 63.
- Annunziato, M.E., et al. 1993. *Bioconjugate Chem.* **4**, 212.
- Caruelle, D., et al. 1988. *Anal. Biochem.* **173**, 328.

## Biotinylation Reagents

CALBIOCHEM® is pleased to present an extensive variety of reagents for the biotinylation of proteins, peptides, glycoproteins, and amino acids. Biotin is a relatively small molecule which can be conjugated to many proteins without altering their biological properties. The strong noncovalent binding between biotin and either avidin or streptavidin and the inherent signal amplification of biotin-avidin systems make biotin a widely used choice for labeling in biochemical and immunological systems. CALBIOCHEM® also offers several derivatives of biotin containing a 6 (X) or 13 (XX) carbon spacer arm to decrease steric hindrance and optimize biotin-avidin complex formation.

### Properties of Biotinylation Reagents

Reagent	Cat. No.	Solubility	Comments	Refs.
Biotin-X-Hydrazide Biotin-XX-Hydrazide	203190-Y 203110-Y	DMSO, CHCl <sub>3</sub> , MeOH	Biotinylates glycoproteins and purified oligosaccharides. Inserts a 6 or 13 atom spacer between biotin and the target ligand. Reacts with the aldehyde groups of periodate-oxidized oligosaccharide moieties to form stable conjugates. When used with carbodiimides, biotin-X-hydrazide can be selectively coupled with carboxyl groups on aspartic and glutamic acid residues.	24-27
Biotin-NHS Biotin-X-NHS Biotin-XX-NHS	203112-Y 203188-Y 203114-Y	DMSO, DMF	Couples biotin to primary amino groups under mild alkaline conditions. Suitable for labeling antibodies, hormones, or polypeptides containing lysine. The 6 or 13 atom spacer arm (X or XX) provides maximum accessibility of the biotin to avidin.	28-30
Biotin-NHS Biotin-X-NHS	203118-Y 203189-Y	Water Water	Water soluble analog of biotin-NHS. Biotinylates the free amino groups of amino acids, peptides, and proteins under mild alkaline conditions. Reacts with primary amines to biotinylate cell surface proteins. The 6 atom spacer (X) helps alleviate steric hindrance between biotin and the target ligand.	31,32
Biotin-X-NHS, Cleavable	203193-Y	Water	Useful for extracting noncovalently associated proteins from complex mixtures. Target proteins with an affinity for a biotinylated macromolecule are isolated using immobilized avidin. The target protein is eluted using a reducing agent to cleave the disulfide bonds.	33
<i>p</i> -Diazobenzoyl biocytin Precursor	286559-Y	DMF, DMSO	After activation to <i>p</i> -diazobenzoyl biocytin, this precursor can be used to label Tyr and His residues. Provides an alternative to NHS-biotin labeling of primary amines.	34
2-Iminobiotin- NHS	401778-Y	DMF, DMSO	Allows direct attachment of iminobiotin to amino-substituted solid supports or protein groups. Imparts a spacer arm of 13.5 Å between the biotin and the primary amine. 2-Iminobiotinylated proteins bind avidin at pH > 9 but are easily eluted at pH 4 or neutral pH with biotin.	35,36
MBB	442631-Y	DMSO	A selective reagent for biotinylating proteins via native or artificially introduced sulfhydryl groups. Provides an alternative to NHS-biotin labeling of primary amines.	37

#### REFERENCES:

24. Kinagwa, T., et al. 1983. *J. Biochem.* **94**, 1160
25. O'Shannessy, D.J., et al. 1987. *Anal. Biochem.* **163**, 204.
26. Reisfeld, A., et al. 1987. *Biochem. Biophys. Res. Commun.* **142**, 519.
27. Bronstein, I., et al. 1990. *Biotechniques* **9**, 160 and 310.
28. Weier, H., et al. 1991. *Biotechniques* **11**, 498.
29. Fabris, F., et al. 1992. *Thrombin Res.* **66**, 409.
30. Smith, J.S., et al. 1991. *Anal. Biochem.* **197**, 247.
31. Broado, R.J., and Pardridge, W.M. 1992. *Bioconjug. Chem.* **3**, 519.
32. Lisanti, M.P., et al. 1989. *J. Cell. Biol.* **109**, 2145.
33. Gretch, D.R., et al. 1987. *Anal. Biochem.* **163**, 270.
34. Wilcheck, M., et al. 1986. *Biochem. Biophys. Res. Commun.* **138**, 872.
35. Hoffmann, K., et al. 1982. *Biochemistry* **21**, 978.
36. Zaheb, R., et al. 1983. *Anal. Biochem.* **129**, 156.
37. Bayer, E.A., et al. 1985. *Anal. Biochem.* **149**, 529.

## Biotin Labeling Kits

Reagent	Cat. No.	Secondary Antibody	Comments
Biotin-NHS Biotin-X-NHS	203113-Y 203187-Y	None	Contains everything needed to label nucleic acids and proteins, including the biotin-NHS ester, buffers, dimethylformamide, and instructions. Includes sufficient label for 110 mg of protein or 5 µmol amino-modified nucleic acid. Biotin-X-NHS includes a 6 atom spacer between biotin and the target ligand that helps alleviate steric hindrance.
Biotin/Streptavidin Immunostaining Kit, Alkaline Phosphatase	568202-Y 568204-Y 568208-Y 568212-Y	Anti-Goat Anti-Human Anti-Mouse Anti-Rabbit	A convenient, rapid, and sensitive method to stain tissues. Kit contains everything needed to stain up to 1000 slides. Includes biotinylated anti-IgG, streptavidin, biotinylated alkaline phosphatase, blocking serum, and instructions.
Biotin/Streptavidin Immunostaining Kit, Peroxidase	568100-Y 568102-Y 568108-Y 568112-Y	None Anti-Goat Anti-Mouse Anti-Rabbit	A convenient, rapid, and sensitive method to stain tissues. Kit contains everything needed to stain up to 1000 slides. Includes streptavidin, biotinylated peroxidase, biotinylated anti-IgG, blocking serum and instructions. Cat. No. 568100-Y does not contain anti-IgG or blocking serum.

### Streptavidin/Avidin Related Products

	Size	Cat. No.
Streptavidin	1 mg	189730-Y
Streptavidin, Alkaline Phosphatase Conjugated	2 ml	189732-Y
Streptavidin, Allophycocyanin Conjugated	1 ml	189735-Y
Streptavidin, Fluorescein Conjugated	1 ml	189734-Y
Streptavidin, β-Galactosidase Conjugated	2 ml	569404-Y
Streptavidin, Peroxidase Conjugated	1 ml	189733-Y
Streptavidin, Phycoerythrin Conjugated	1 ml	189737-Y
Streptavidin, TEXAS RED® Conjugated	1 ml	189738-Y
Streptavidin-Coated Immunomagnetic Beads	2 ml	400996-Y
Avidin, Egg White	10 mg	189725-Y
Avidin, Alkaline Phosphatase Conjugated	2 ml	189726-Y
Avidin, Ferritin Conjugated	5 mg	189752-Y
Avidin, Fluorescein Conjugated	1 ml	189727-Y
Avidin, Peroxidase Conjugated	2 ml	189728-Y
Avidin, Rhodamine Conjugated	2 ml	189743-Y
Avidin-Agarose	5 ml	189742-Y

TEXAS RED® is a registered trademark of Molecular Probes.

***CALBIOCHEM® offers an extensive collection of enzyme-conjugated antibodies and purified enzymes for your research needs. Please call our Technical Service Department or your local distributor and ask for your free copy of "Enzyme-Linked Detection Systems."***

Please call our Technical Service Department or your local sales office for more information on these products.

**United Kingdom**  
Tel (0115) 943 0840  
Fax (0115) 943 0951  
customer.service@cnuuk.co.uk

**USA, Canada, & Mexico**  
Tel (800) 854-3417  
Fax (800) 776-0999  
technical@calbiochem.com

**Germany**  
Tel (06196) 63955  
Fax (06196) 62361  
customer.service@calbiochem-novabiochem.de