

Modifying agents are used to modify the biomolecules reactivity prior to cross-linking, to change their isoelectric point modifying their interactions, to reduce non specific binding, to change their structure for structure or function analysis, ... Refer also to Cross-linking technical tips (page B11). They are categorized in oxido-reducers, cleavage agents (chemical or enzyme reaction), conversion of chemical group and specialties. Reagents to quantitate target groups are finally presented.

All modifier agents are referred in the table of cross-linkers/modifiers, page B15. Only important chemical modifiers are presented below.

Oxido-Reducing agents

Oxido-Reduction of biomolecules include :

♦ **Reduction of disulfide bridges** (-SS-) (i.e. from proteins) to free SH. It is required for modification of biomolecules before biochemical reactions, i.e. oriented cross-linking. Standard reducers are, by order of decreasing reducing strength, TCEP>DTT>ME>2MEA>cysteine . As -SS- bridges are essential to protein tertiary structure and free SH are often involved in biological activity (i.e. enzymatic site), thio-reduction is also important for the protein structure and function study.

♦ **Reduction of Carboxyls** (-COOH) to aldehyde (CHO). It is also required in biochemistry, to introduce reactive groups (-CHO) in proteins or carbohydrates. NaBH₄ is commonly used for protein coupling.

♦ **Oxidation of carbohydrates** . Metaperiodate is commonly used for oxidizing antibody glycones before peroxidase coupling (Nakane technique).

Dithiothreitol

see description product **UP284250** page B35

TCEP

see description product **UP284250** page B35

Glutathione

MW : 307.33

Glutathione is an important and potent water-phase antioxidant, and an essential cofactor for antioxidant enzymes

Description	Cat.#	Qty
Glutathione, Reduced	288283	50 g

Cysteine

Description	Cat.#	Qty
Cysteine HCl, UltraPure	00979L	250 g

Sodium cyanoborohydride

MW : 62.84

Toxic

Forms stable bonds between aldehyde- and amine-containing molecules, enabling efficient labeling, conjugation and biomolecules immobilization.

Description	Cat.#	Qty
Sodium cyanoborohydride 95%	05777C	10 g

Sodium borohydride

MW : 37.83

Description	Cat.#	Qty
Sodium borohydride 98+%, powder	07998H	5 g

Chemical cleavage

Proteins may be cleaved at specific site by chemical reaction.
Most useful cleavage sites are :

Cleavage site		Product	Applications / Feature
Cys-Cys	Disulfide bond	DTT TCEP	Dissociation of polymeric proteins and complexes Denaturation of proteins ◆ Before electrophoresis analysis (i.e. SDS-PAGE) ◆ Before labeling (i.e. with Thiol reactive biotinylation agents) ◆ Before making a conjugate (i.e. with Cross-linkers)
Cys	Cystein	FeBABE	3D-Structure analysis of protein complexes
Try	Tryptophane	BNPS	Specific amino acid cleavage
Arg	Arginine	APG, HPG	Specific amino acid cleavage

BNPS-Skatole

2-(2'-Nitrophenylsulfonyl)-3-methyl-3-bromindolenine

MW : 363.2

Cleaves tryptophan residues in peptidic chains

Applications : Hunziker (1980), Fernandez (1985), Fontana (1972)

Description	Cat.#	Qty
BNPS-Skatole	UP20955A	1 g

DTT (Cleland's reagent)

DL-Dithiothreitol

MW : 154.25

An excellent reducing reagent of disulfides bridges in proteins

- ◆ Replaces advantageously the pungent b-mercaptoethanol
- ◆ Ultrapure grade (>99.4% SH form)

Applications :

Reduction of samples before SDS-PAGE

Reduction of dimeric Cys-containing peptides (Zahler 1983)

Related product SH can be dosed with DTNB #UP01566

Description	Cat.#	Qty
DTT(Cleland's reagent)	UP284250	1 g
	UP284255	5 g

FeBABE

Cleavage of the vicinal Cys residues. Great for 3D structure analysis of protein complexes (See complete description page B223)

Description	Cat.#	Qty
FeBABE	UP99476	

TCEP

Tris(2-carboxyethyl)phosphine hydrochloride

MW : 286.65

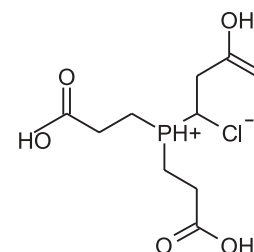
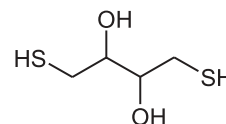
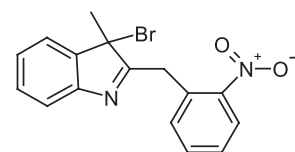
Solubility : > 310g/L

Replaces DTT for most exigent requirements

- ◆ Reduces completely and selectively alkyl disulfides in mild conditions : does not react with buried sulfhydryls or other chemical moieties
- ◆ Works in a broad range of pH (7-9) and better than DTT at low pH
- ◆ Water-soluble and resistant to air oxidation
- ◆ Odorless

Applications : Reduction of biomolecules for structure studies, conjugations

Description	Cat.#	Qty
TCEP	UP242214	1 g



See also :

APG # UP28071

Arginine specific cross-linking. See page B28

HPG # UP36862

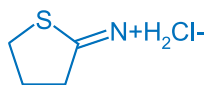
Arginine modification. See page B36

DTNP # UP01566

SH modification and quantitation. See page B39

Isolation/Modification/Labeling

Modifying Agents



Chemical Conversion of Chemical groups

2-Iminothiolane (Traut's reagent)

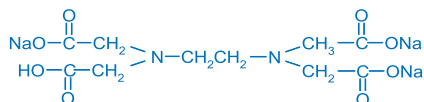
2-iminothiolane.HCl

MW : 137.6

Converts amines to sulfhydryls

- ◆ Reacts with free SH at pH7-10 in water
- ◆ Useful for designing oriented conjugates

Description	Cat.#	Qty
2-Iminothiolane Hydrochloride	UP42425B	1 g
	UP42425A	500 mg



EDTA

Chelating agent of divalent ions

Description	Cat.#	Qty
EDTA	UP03629	

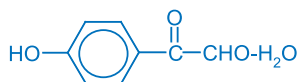
HPG

β-Hydroxyphenyl Glyoxal

MW : 168.2

Modify arginine residues

- ◆ Reacts in mild conditions (pH7-9)
- ◆ Arginine specific
- ◆ Can be monitored at 340 nm
- ◆ More resistant than p-Nitrophenyl Glyoxal
- ◆ More soluble than phenylglyoxal



Description	Cat.#	Qty
HPG	UP36862A	100 mg

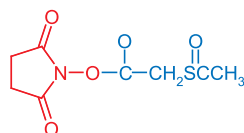
SATA

N-succinimidyl S-acetylthioacetate hydrochloride

MW : 231.2

Converts amines to protected sulfhydryl

- ◆ Prevents disulfide formation (ex. peptides)
- ◆ Easy deprotection in mild conditions



Applications : Can also be used as a cross-linking agent by biomolecule thiolation then exchanging sulfhydryl with another molecule.

Description	Cat.#	Qty
SATA	UP84235A	100 mg

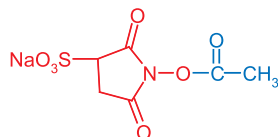
SulfoNHS- acetate

Sulfo-succinimidyl-acetate

MW : 259.2

Blocks primary amines

- ◆ Water-soluble
- ◆ Efficient at pH7-10



Applications : peptide or proteins modification Binding site studies

Description	Cat.#	Qty
SulfoNHS- acetate	UP69380A	100 mg

NHS 8 Sulfo-NHS

Used to activate COOH

Description	Cat.#	Qty
Sulfo-NHS	FP544225	100 mg
NHS	UP04594A	500 mg
MW : 115.09		

See also Hydrazone modifiers page B31

Modifying agents – Application Specialties

FeBABE

β -BromoAcetamidoBenzyl-EDTA Iron (III) chelate

A unique tool to study tridimensional structures!

- ◆ Binds to Cys-SH residue then cleaves peptide or DNA chain within 12 Å vicinity
- ◆ Quick reaction
- ◆ Bromoacetamide group binds to free sulfhydryls, and introduces an Iron chelate. The chelate cleaves spatially close DNA or peptidic bond within 10 sec to 20 min in presence of ascorbic acid and H_2O_2 .

Applications : Elucidation of 3 dimensionnal structures (E. Coli cytochrome bd quinol oxidase, a subunit of E.Coli polymerase...). Used too as a chemical nuclease.

Description	Cat.#	Qty
FeBABE	UP994760	1 mg



Related products :

Reducing agents DTT #UP28425, TCEP #UP242214

For desalting the excess of reagent see Dialysis (Uptispin)

MTSEA

2-Aminoethyl MethaneThioSulfonate Hydrobromide

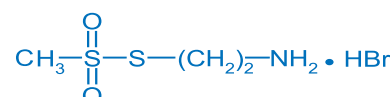
- ◆ Alkylthiosulfonate reacts on sulfhydryls

Useful in the membrane proteins mapping, as ion channels and proteins transport, as well as enzymes and receptors

This reagent would fit inside a cylinder about 0.6nm in diameter and 1nm in length (Akabas 1992).Exists also as (trimethylammonium)ethyl (MTSET U0351), sulfonatoethyl (MTSES U0350) derivatives, and MTSEA-Biotin derivate probe (UPR5762).The use of charged alkylthiosulfonate reagents when combined with site-specific introduction of cysteines can provide information about the structure and function of ion channels (SCAM method) and transports proteins, as well as enzymes and receptors.

Literature : Akabas M.H., Stauffer D.A., XU M. and Karlin A., Science 1992, 258, 307-310 Akabas M.H., Kaufman C., Archdeacon P., and Karlin A., Neuro 1994, 13, 919-927. Karlin A. and Akabas M.H., in Methods Enzymol. 1998, 293, 123-136 Kenyon G.L. and Bruce T.W., in Methods Enzymol., 1977, 407-430 Stauffer D.A. and Karlin A., Biochem 1994, 33, 6840-6849

Description	Cat.#	Qty
MTSEA	UP99618	100 mg
	UP99619	500 mg



Enzyme Modifying Agents

Papain

Papain is a thiol protease which requires a mild reducing environment for activity. Thus cleavage is carried out in the presence of thiol compounds, such as cysteine or mercaptoethanol. It is used typically to scavenge the Fc fragments and any undigested IgG molecules. Cleavage occurs in the hinge region of the IgG molecule, yielding Fab fragments and Fc fragments. The Fab fragment is monovalent, and therefore cannot stimulate antigen cross-linking or precipitation.

Description	Cat.#	Qty
Papain - Activity > 46000 USP/NF U/mg	14542B	10 g

Immobilized Papain

Immobilized Papain ensures high protease activity and leaves your sample free of papain.

Description	Cat.#	Qty
Immobilized Papain	414645	10 ml

Isolation/Modification/Labeling

Modifying Agents

Pepsin

It is typically used to cleave IgG antibodies into F(ab)₂ fragments. Note : IgG2b, IgG3, and IgG4 are particularly sensitive, while IgG1 and IgG2a are relatively resistant to pepsin by nature, so they are usually treated by a short preliminary incubation at pH 2.8 for full conversion. See also description pepsinin "cell culture" pepsin inhibitors (pepstatin) page E17.

Description	Cat.#	Qty
Pepsin - Activity : 1:3000 - 1:3500	FM2178	250 g

Immobilized Pepsin

Immobilized Pepsin ensures high protease activity and leaves your sample free of pepsin.

Description	Cat.#	Qty
Immobilized Pepsin - 3-4 mg pepsin/ml	UP499785	10 ml

Proteinase K

See description page D18 section "genomics".

Description	Cat.#	Qty
Proteinase K - Activity : > 30 U/mg	858700	100 mg

Trypsin

See description page E29 section section "cell culture", and trypsin inhibitors pages E16 - E19.

Description	Cat.#	Qty
Trypsin - Activity : >2500 U Trypsin/mg	N15150	1 g

Quantification of chemical groups (SH, NH₂, Biotin)

Following reagents are usefull to quantify chemical groups before conjugations.

DTNB (Ellman's reagent)

5-5'-DiThiobis-(2-NitroBenzoic) acid

MW : 396.36

A sensitive reagent for the sulfhydryls quantitation

- ◆ Useful for monitoring SH content in peptides and proteins before and after reaction of SH-reactive agents
- ◆ Very strong detection is measured at 412 nm

Description	Cat.#	Qty
DTNB (Ellman's reagent)	UP01566H	1 g
	UP01566I	5 g

HABA

2-Hydroxyazobenzen-4'-Carboxylic Acid

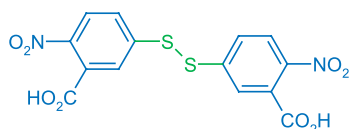
MW : 242.24

Colorimetric quantitation of biotin

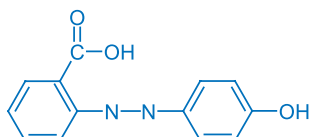
- ◆ Works over a wide range of pH and salt concentration
- ◆ Works for both purified and complex samples

The HABA when binding to avidin, produces a yellow-orange colored complex which absorbs at 500 nm. Biotin displaces easily the HABA from the complex, causing the absorbance to decrease. The biotin present in a sample can thus be determined by interpolation from a standard curve of free biotin, then the number of moles of biotin per mole of biotinylated protein can be calculated.

Description	Cat.#	Qty
HABA	UP05361D	1 g



Related product :
N-Acetyl-L-Cysteine, Ultrapure, UP04901V (1 g)
MW163
AcCys is used as a sulfhydryl standard for the DTNB method



Related product :
Avidin cat.# UP39860
Biotin cat.# UP10685

B.38



OPA powder

o-Phthalaldehyde

MW : 134.1

Reagent for Amines Detection and Quantitation

OPA is reactive toward amines to form an unstable complex. The complex can be detected spectrophotometrically at 560 nm, or by fluorescence (exc.@330-390 nm, emission@436-475 nm). Uptima offers a ready-to-use and stable formulation with a complete protocole to quantitate amines in proteins and peptides, or any other biochemical bearing primary or secondary amines.

Description	Cat.#	Qty
OPA powder, Ultrapure	UP02727A	1 g

Sulfo, SDTB

SulfoSuccinimidyl-4-o-(4,4-DimethoxyTrityl)-Butyrate

MW : 605

Detect immobilized amines

Description	Cat.#	Qty
Sulfo,SDTB	UP35300A	50 mg

4NBA

(4-Nitrobenzaldehyde)

Quantitate hydrazine and hydrazide groups.

Description	Cat.#	Qty
4NBA	BL9650	100 mg

2HP

(2-Hydrazinopyridine dihydrochloride)

MW : 182.1

Quantitate aldehyde groups.

Description	Cat.#	Qty
2HP	O19022	100 mg

2SBA

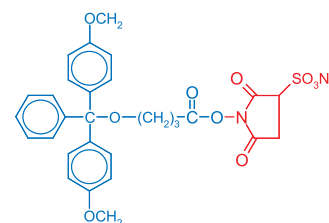
(2-Sulfobenzaldehyde)

MW : 208.2

Quench or cap hydrazone conjugation reactions.

Description	Cat.#	Qty
2SBA	A42050	100 mg

Related product
N-Acetyl-L-Lysine, Ultrapure,
MW188, UP09111A, (1g) Ac-Lys is used as an amine standard for the OPA method. See also OPA protein assay #51225A, 1 kit



*See also section "Hydrazone chemistry" page B31

*See also derivatization reagents for aa, peptides, and proteins

Isolation/Modification/Labeling

Modifying Agents

Biotinylation reagents

Product			Spacer		Cleavability	Cleavability	Water Solubility	Iodinated	Functional group(s)			Cleavability
Name	Cat.#	MW	Type	Length					group 1	group 2	group 3	
AMCH-Biotin	UPR0756A	341.40	alkyl		No	No	.		/CHO		Biotin	B48
Aminoethyl-SS-Biotin	BT3761	382.97	Linear	7 Atoms	Thiols	Thiols			SH §		Biotin	B49
Biotin-4-fluorescein	UP99687A	523.60	alkyl	6 Atoms	No	No	.		MAL		Biotin	B46
Biotin-Chromalink-NHS	M1769A	644.71	alkyl	31.62 Ang.	No					FluoLabel	Biotin	B50
Biotin-PEO3-BP	BT3601	810.92	PEO	20 Atoms	No	No	Yes		NHS	FluoLabel	Biotin	B43
Biotin-PEO3-BP	NI0231	654.82	PEO	16.9 Ang.	No	No	Yes	No	BP		Biotin	
Biotin-PEO3-Cyanocobalamin	BJ0111	473.61	PEO	-	No	No	Yes	No		FluoLabel	Biotin	
Biotin-G-PEO4-COOH	BJ007A	491.60	PEO	19.2 Ang.	No	No	High	No	COOH		Biotin	B48
Biotin-Ic-COOH	BT3641	357.48	alkyl	6 Atoms	No	no		No	COOH		Biotin	B48
Biotin-Ic-COOH	U14681	357.47	alkyl	6 Atoms	No	no		No	COOH		Biotin	B48
Biotin-Ic-Ic-COOH	BT3651	421.61	alkyl	12 Atoms	No	no		No	COOH		Biotin	B48
Biotin-Ic-NH2	BT3701	342.51	alkyl	6 Atoms	No	no		No	NH2		Biotin	
Biotin-X-Cadaverine	UP84961A	555.67	alkyl	6 Atoms	No	no		No	NH2		Biotin	B49
Biotin-MTSEA	UPR5752	381.52	alkyl		No	No		No	MTS		Biotin	B47
Biotin	UP10685E	244.31	-	-	-			No	COOH		Biotin	B48
Biotin Amido PentylAmine (BAPA)	UP84961A	328.48	Linear	5 Atoms	No	No		No	NH2		Biotin	B49
Biotin-NH2	BT3701	286.39	alkyl	2 Atoms	No	no		No	NH2		Biotin	
Biotin-PDA	BT3631	412.60	alkyl		No	no		No	PDS		Biotin	B46
Biotin-PEO3-Amine	77872A	418.55	PEO	20.4 Ang.	No	no	Yes	No	NH2		Biotin	B49
Biotin-PEO4-Amine	UP91577A	418.56	PEO	22.9 Ang.	No	no	Yes	No	NH2		Biotin	B49
Biotin-PEO6-Biotin	Q7467A	732.92	PEO	43.4 Ang.	No	no	Yes	No		Biotin	Biotin	B49
Biotin-PEO12-Biotin	UPT5046A	637.81	Arom	19 Atoms	No	no	Yes	No		Biotin	Biotin	B49
Biotin-PEO3-Fluorescein	BT3721	720.80	PEO	8 Atoms	No	no	Yes	No		FluoLabel	Biotin	B50
Biotin-PEO3-Maleimide(II)	BT3751	454.54	PEO	8 Atoms	No	no	Yes	No	MAL		Biotin	
Biotin-PEO4-ANB	BT3741	608.68	PEO	12 Atoms	No	no	Yes	No	NPA		Biotin	B45
Biotin-PEO4-Hydrazide	BJ008A	505.63	PEO	20.6 Ang.	No	No	Yes	No	HYD		Biotin	B47
Biotin-PEO4-Maleimide	UPR2028A	505.63	PEO	38 Ang.	No	no	Yes	No	MAL		Biotin	B45
Biotin-PEO-CO2-NHS	422850		PEO		No	No	High	No	NHS		Biotin	
Biotin-PEO4-TFPA	BT3621	635.64	PEO	33 Ang.	No	no	Yes	No	TFPA		Biotin	B45
Biotin-rhodamine	AM5541	802.94	alkyl		No					FluoLabel	Biotin	B50
BMCC-Biotin	UP27443A	533.7	alkyl	32.6 Ang.	No	No		Yes	MAL		Biotin	B46
Fluorescein-Ic-biotin	FP-959145	831.03	alkyl		No					FluoLabel	Biotin	B50
a-Fmoc-L-Lys(+)-Biotinyl-OH	BT3691	594.78	alkyl	6 Atoms	No	no		No	OH	NH2 §	Biotin	
a-Fmoc-L-Lys(+)-Biotinyl-e-aminocaproyl-OH	BT3681	707.90	alkyl	16 Atoms	No	no		No	OH	NH2 §	Biotin	
HPDP-Biotin	UP83035A	539.79	alkyl	29.2 Ang.	No	No	No	.	PThio		Biotin	B46
Hydrazide-biotin	22772A	386.52	alkyl		No		No		HYD		Biotin	B47
Hydrazide-Biotin	UP36466	258.30	alkyl	4 Atoms	No	No	No	.	HYD		Biotin	B47
Hydrazide-Ic-Biotin	UP78631	371.50	alkyl	12 Atoms	No	No	No	.	HYD		Biotin	B47
Hydrazide-Ic-Ic-Biotin	BT3671	441.64	alkyl	14 Atoms	No	No	No	.	HYD		Biotin	B47
Iminobiotin	UP39375A	244.33	-		No		No		COOH		Biotin	B48
IodoAcetyl-Biotin	UP55533A	510.40	alkyl	27.1 Ang.	No	No	No	.	HYD		Biotin	B46
Maleimido-Biotin	UP48198A	451.00	alkyl		No	No	No	.	MAL		Biotin	B45
Maleimide-PEO3-Biotin	UP87284A	525.62	PEO	29.1 Ang.	No	No	No	.	MAL		Biotin	B45
Maleimide-PEO4-Biotin	UPAK789A	597.73	PEO	24.9 Ang.	No	No	No	.	MAL		Biotin	
NHS-Biotin	UP39044A	341.38	alkyl	13.5 Ang.	No	No	No	.	NHS		Biotin	B43
SulfoNHS-Biotin	UP52117A	443.42	alkyl	13.5 Ang.	No	No	Yes	.	NHS(s)		Biotin	B43
NHS-Imino-Biotin	UP35329A	340.38	alkyl	13.5 Ang.	No	No	No	.	NHS		Biotin	B44
NHS-Ic-Biotin	UP85262A	455.55	alkyl	22.4 Ang.	No	No	No	.	NHS		Biotin	B43
SulfoNHS-Ic-Biotin	UP54398A	556.58	alkyl	22.4 Ang.	No	No	Yes	.	NHS(s)		Biotin	B43
NHS-Ic-Ic-Biotin	UP37924A	567.71	alkyl	30.5 Ang.	No	No	No	.	NHS		Biotin	B44
SulfoNHS-Ic-Ic-Biotin	UP29847A	669.75	alkyl	30.5 Ang.	No	No	Yes	.	NHS(s)		Biotin	B44
NHS-PEO4-Biotin	UPR2027A	588.67	PEO	24.9 Ang.	No	No	High	.	NHS		Biotin	B42
NHS-SS-Biotin	UPS0738A	504.65	alkyl	24.3 Ang.	Yes		No	.	NHS		Biotin	B44
SulfoNHS-SS-Biotin	UP53031A	606.69	alkyl	24.3	Thiols	Thiols	Yes	.	NHS(s)		Biotin	
PFP-Biotin	UP88364A	410.37	alkyl	9.6 Ang.	No	No	No	.	PFP		Biotin	B45
Psoralen-PEO4-Biotin	UPL7784A	688.80	PEO	36.9 Ang.	No	No	Yes	.		Psoralen	Biotin	B48

Legend : See page B19.