

Cat #	Product Name	Unit Size
P-21879	Pro-Q™ Oligohistidine Blot Stain Kit #2 *with biotin NTA, streptavidin and ELF® 39 phosphate* *20 minigel blots*	1 kit
P-21876	Pro-Q™ Sapphire 365 oligohistidine gel stain *20 minigels*	500 mL
P-21877	Pro-Q™ Sapphire 488 oligohistidine gel stain *20 minigels*	500 mL
P-21863	Pro-Q™ Western Blot Stain Kit #1 *with goat anti-mouse IgG and DDAO phosphate* *>20 minigel blots*	1 kit
P-21860	Pro-Q™ Western Blot Stain Kit #2 *with goat anti-mouse IgG, DDAO phosphate and SYPRO® Ruby protein blot stain* *10-20 minigel blots*	1 kit
P-21864	Pro-Q™ Western Blot Stain Kit #3 *with goat anti-rabbit IgG and DDAO phosphate* *>20 minigel blots*	1 kit
P-21861	Pro-Q™ Western Blot Stain Kit #4 *with goat anti-rabbit IgG, DDAO phosphate and SYPRO® Ruby protein blot stain* *10-20 minigel blots*	1 kit
P-21865	Pro-Q™ Western Blot Stain Kit #5 *with streptavidin and DDAO phosphate* *>20 minigel blots*	1 kit
P-21862	Pro-Q™ Western Blot Stain Kit #6 *with streptavidin, DDAO phosphate and SYPRO® Ruby protein blot stain* *10-20 minigel blots*	1 kit
P-6649	Protein molecular weight standards *broad range* *200 gel lanes*	400 µL
S-6656	SYPRO® photographic filter	each
S-11791	SYPRO® Ruby protein blot stain *10-40 blots*	200 mL
S-12000	SYPRO® Ruby protein gel stain	1 L
S-12001	SYPRO® Ruby protein gel stain	200 mL
S-21900	SYPRO® Ruby protein gel stain *bulk packaging*	5 L
S-12010	SYPRO® Tangerine protein gel stain *5000X concentrate in DMSO*	500 µL

9.5 Reagents for Peptide Analysis, Sequencing and Synthesis

This section describes Molecular Probes' reagents used in the synthesis of fluorescent dye- or hapten-labeled peptides and fluorogenic protease substrates, as well as in peptide and protein sequencing. The dominant chemistry for sequencing peptides employs the nonfluorescent reagent, phenyl isothiocyanate, which forms phenylthiohydantoin (PTH) in the sequencing reaction. Some of our fluorescent probes and research chemicals have been used for N-terminal amino acid analysis and peptide sequencing, as well as for protein fragment modification prior to PTH sequencing.

N-Terminal Amino Acid Analysis

Except when it is already blocked by formylation, acetylation, pyroglutamic acid formation or other chemistry, the N-terminal amino acid of proteins can be labeled with a variety of fluorescent and chromophoric reagents from Chapter 1. However, only those functional groups that survive complete protein hydrolysis, such as sulfonamides, are useful for N-terminal amino acid analysis. Dansyl chloride (D-21) and dabsyl chloride (D-1537) are the most commonly employed reagents for such analyses.¹⁻³

Nonacylated N-terminal serine and threonine residues of proteins can be periodate-oxidized to aldehydes⁴⁻⁶ (Figure 3.1) that can then be modified by a variety of hydrazine derivatives listed in Section 3.2. Only peptides and proteins that contain these two terminal amino acids become fluorescent, although oxidation of the carbohydrate portion of glycoproteins to aldehydes may cause interference in this analysis.

N-Acetylated or N-formylated proteins have been detected by transfer of the acyl group to dansyl hydrazine (D-100) and subsequent chromatographic separation of the fluorescent product.^{7,8}

The sensitivity of this method can likely be improved by the use of other fluorescent hydrazine derivatives described in Section 3.2.

Peptide Sequencing

As analogs of phenyl isothiocyanate, the peptide conjugates of fluorescein-5-isothiocyanate (FITC, F-143, F-1906, F-1907; Section 1.5), and other fluorescent isothiocyanates are susceptible to Edman degradation via their thiohydantoin. Thus, these fluorescent reagents are potentially useful for ultrasensitive amino acid sequencing.⁹⁻¹²

Peptide Synthesis

Peptides specifically labeled with fluorescent dyes, haptens, photoactive groups or radioisotopes are important both as probes for receptors and as substrates for enzymes (Section 10.4). Labeled peptides can be prepared by modifying isolated peptides or by incorporating the label during solid-phase synthesis. Molecular Probes offers some fluorescent neuropeptides, most of which are described in Section 16.2.

Labeling Peptides in Solution

Appropriately substituted synthetic peptides can be labeled in solution by almost any of the reactive probes in Chapters 1-5 (see Labeling Small Peptides with Amine-Reactive Dyes in Organic Solvents). Many peptides contain multiple residues that can be modified, potentially leading to complex mixtures of products, some of which may be biologically inactive. Modification of the peptide's thiol group by one of the thiol-reactive reagents described in Chapter 2 is usually easy, selective and very efficient.

If the peptide is synthetic, or can be modified by site-directed mutagenesis, then incorporation of a cysteine residue at the desired site of labeling is recommended. The N-terminus of peptides, which has a lower pK_a than the ϵ -amino group of lysine residues, can sometimes be labeled in the presence of other amines if the pH is kept near neutral. Conversion of tyrosine residues to *o*-aminotyrosines (Section 3.1, Figure 3.3) can be used to provide selective sites for peptide modification, unless the tyrosine residues are essential for the biological activity of the peptide.

Solid-Phase Synthesis of Labeled Peptides

Because specific labeling of peptides in solution is problematic, it may be more convenient to conjugate the fluorophore to the N-terminus of a resin-bound peptide *before* removal of other protecting groups and release of the labeled peptide from the resin. About five equivalents of an amine-reactive fluorophore are usually used per amine of the immobilized peptide. The fluorescein, eosin, Alexa Fluor, Oregon Green, Rhodamine Green, tetramethylrhodamine, Rhodamine Red, Texas Red, coumarin and NBD fluorophores, the QSY, dabcyI and dabSYI chromophores and biotin are all expected to be reasonably stable to hydrogen fluoride (HF), as well as to most other acids.^{13–18} These fluorophores, chromophores and biotin are also expected to be stable to reagents used for deprotection of peptides synthesized using Fmoc chemistry.¹⁹ The BODIPY fluorophore may be unstable to the conditions used to remove some protecting groups.

Molecular Probes has prepared some unique reagents for automated synthesis of peptides that are specifically labeled with fluorophores, chromophores and haptens. Use of these precursors permits the incorporation of these groups at specific sites in the peptide's sequence. The α -Fmoc derivative of ϵ -dabcyI-L-lysine (D-6216) can be used to incorporate the dabcyI chromophore at selected sites in the peptide sequence. The dabcyI chromophore, which has broad visible absorption (Figure 10.46), has been extensively used as a quenching group in the automated synthesis of HIV protease (H-2930, Section 10.4), renin (R-2931, Section 10.4) and other fluorogenic peptidase substrates.^{20–23} The dabcyI group can also be incorporated at the N-terminus by using dabcyI succinimidyl ester^{18,24} (D-2245). The aminonaphthalene derivative EDANS (A-91) has been the most common fluorophore for pairing with the dabcyI quencher in fluorescence resonance energy transfer (FRET, see Section 1.3) experiments because its fluorescence emission spectrum overlaps the absorption spectrum of dabcyI (Figure 10.46). This fluorophore is conveniently introduced during automated synthesis of peptides by using γ -EDANS- α -Fmoc-L-glutamic acid (F-11831) or the corresponding *t*-BOC derivative^{18,20} (B-6215). The tetramethylrhodamine fluorophores can be incorporated during automated Fmoc synthesis of peptides using our single-isomer α -(Fmoc)- ϵ -TMR-L-lysine building block (F-11830). Site-selective biotinylation of peptides can be achieved using the Fmoc derivative of biocytin (B-20651) during automated synthesis. This reagent can also be attached to the synthesis resin as the first residue to provide for automated synthesis of C-terminal biotinylated peptides.

Our QSY dyes (Section 1.6, Section 1.8) have broad visible to near-infrared absorption (Figure 1.66, Table 1.7). These dyes, which are essentially nonfluorescent, are particularly useful as energy

acceptors from blue-, green-, orange- or red-fluorescent donor dyes (Table 1.8). The QSY 7, QSY 9, QSY 21 and QSY 35 chromophores can be conjugated to amines via their succinimidyl esters (Q-10193, Q-20131, Q-20132, Q-20133). The QSY 7 dye can also be conjugated to thiols of peptides via its maleimide (Q-10257) and the QSY 35 dye coupled via its iodoacetamide (Q-20348). Additionally, peptide amides can be prepared from the QSY 7 and QSY 35 aliphatic amines (Q-10464, Q-20540). We have also prepared α -(Fmoc)- ϵ -QSY 7-L-lysine and α -Fmoc- β -QSY 35-L-alanine (Q-21930, Q-21931), which can be used in the automated synthesis of QSY 7 quencher- or QSY 35 quencher-containing peptides.

References

1. J Chromatogr 553, 123 (1991); 2. Anal Biochem 174, 38 (1988); 3. Anal Biochem 170, 542 (1988); 4. Biochem J 108, 883 (1968); 5. Biochem J 95, 180 (1965); 6. Biochem J 94, 17 (1965); 7. J Cell Biol 106, 1607 (1988); 8. Anal Biochem 29, 186 (1969); 9. Biosci Biotechnol Biochem 58, 300 (1994); 10. Biol Chem Hoppe Seyler 367, 1259 (1986); 11. FEBS Lett 198, 150 (1986); 12. Anal Biochem 141, 446 (1984); 13. Biochemistry 33, 7211 (1994); 14. Biochemistry 33, 6966 (1994); 15. J Biol Chem 269, 15124 (1994); 16. Techniques in Protein Chemistry V, Crabb JW, Ed. pp. 493–500 (1994); 17. Anal Biochem 202, 68 (1992); 18. J Med Chem 35, 3727 (1992); 19. Biochemistry 33, 10951 (1994); 20. Bioorg Med Chem Lett 2, 1665 (1992); 21. J Protein Chem 9, 663 (1990); 22. Science 247, 954 (1990); 23. Tetrahedron Lett 31, 6493 (1990); 24. FEBS Lett 297, 100 (1992).

TECHNICAL NOTE

Labeling Small Peptides with Amine-Reactive Dyes in Organic Solvents

Most of the product literature associated with our amine-reactive dyes provides protocols for labeling proteins, typically IgG antibodies in aqueous buffers. The following protocol is a starting point for labeling peptides in organic solvents. Please note that the reaction conditions, including concentrations of the reactants and the reaction times, may require optimization. Furthermore, many peptides are not soluble in a 100% organic solution. It is very important to test the solubility of the peptide in DMSO or DMF before attempting this procedure.

1. Dissolve the peptide to be labeled in DMSO or DMF at 0.1–1 mM.
2. Add 100 mM triethylamine to the reaction solution. This will ensure that the amines to be derivatized are deprotonated.
3. Add the amine-reactive dye to the reaction solution. The reactive dye should be in a 1:1 to 3:1 molar ratio to the peptide.
4. React at room temperature or at 4°C for at least 4 hours with continuous stirring, protected from light. The reaction can proceed overnight. Thin-layer chromatography may be useful for monitoring the reaction's progress.
5. Purify the conjugate by an appropriate method, such as HPLC-based separation.

Data Table — 9.5 Reagents for Peptide Analysis, Sequencing and Synthesis

Cat #	MW	Storage	Soluble	Abs	EC	Em	Solvent	Notes
A-91	288.30	L	pH >10, DMF	335	5,900	493	pH 8	
B-6215	495.55	F,D,L	DMF	341	5,400	470	MeOH	
B-20651	594.72	F,D	DMF, MeCN	<300		none		
D-21	269.75	F,DD,L	DMF, MeCN	372	3,900	none	CHCl ₃	1, 2
D-100	265.33	L	EtOH	336	4,400	534	MeOH	
D-1537	323.80	F,DD,L	DMF, MeCN	466	33,000	none	MeOH	2, 3
D-2245	366.38	F,D,L	DMF, DMSO	453	32,000	none	MeOH	3
D-6216	619.72	F,D,L	DMF, MeCN	427	30,000	none	MeOH	
F-11830	780.88	F,D,L	DMF, MeCN	543	92,000	570	MeOH	
F-11831	617.67	F,D,L	DMF, MeCN	341	5,200	471	MeOH	
Q-10193	791.32	F,D,L	DMSO	560	90,000	none	MeOH	
Q-10257	858.45	F,D,L	DMSO	560	92,000	none	MeOH	
Q-10464	814.87	L	DMSO	560	92,000	none	MeOH	
Q-20131	951.43	F,D,L	H ₂ O, DMSO	562	88,000	none	MeOH	4
Q-20132	815.34	F,D,L	DMSO	661	90,000	none	MeOH	
Q-20133	411.33	F,D,L	DMSO	475	23,000	none	MeOH	
Q-20348	453.20	F,D,L	DMSO	475	24,000	none	MeOH	5
Q-20540	399.29	L	DMSO, DMF	472	24,000	none	MeOH	
Q-21930	1044.66	F,D,L	DMF, MeCN	560	90,000	none	MeOH	
Q-21931	565.54	F,D,L	DMF, MeCN	475	23,000	none	MeOH	

For definitions of the contents of this data table, see "How to Use This Book" on page viii.

Notes

- D-21 butylamine derivative has Abs = 337 nm (EC = 5300 cm⁻¹M⁻¹), Em = 492 nm in CHCl₃. Em and QY are highly solvent dependent: Em = 496 nm (QY = 0.45) in dioxane, 536 nm (QY = 0.28) in MeOH and 557 nm (QY = 0.03) in H₂O (Biochemistry 6, 3408 (1967)). EC typically decreases upon conjugation to proteins (EC = 3400 cm⁻¹M⁻¹ at 340 nm) (Biochemistry 25, 513 (1986)). Fluorescence lifetimes (τ) of protein conjugates are typically 12–20 nanoseconds (Arch Biochem Biophys 133, 263 (1969); Arch Biochem Biophys 128, 163 (1968)).
- Do NOT dissolve in DMSO.
- D-1537 reaction product with butylamine: Abs = 435 nm (EC = 31,000 cm⁻¹M⁻¹), nonfluorescent in MeOH. D-2245 reaction product with butylamine: Abs = 428 nm (EC = 32,000 cm⁻¹M⁻¹), nonfluorescent in MeOH.
- This sulfonated succinimidyl ester derivative is water-soluble and may be dissolved in buffer at ~pH 8 for reaction with amines. Long-term storage in water is NOT recommended due to hydrolysis.
- Iodoacetamides in solution undergo rapid photodecomposition to unreactive products. Minimize exposure to light prior to reaction.

Product List — 9.5 Reagents for Peptide Analysis, Sequencing and Synthesis

Cat #	Product Name	Unit Size
A-91	5-((2-aminoethyl)amino)naphthalene-1-sulfonic acid, sodium salt (EDANS)	1 g
B-20651	ε-biotinoyl-α-(9-fluorenylmethoxycarbonyl)-L-lysine (FMOC biocytin)	25 mg
B-6215	5-((2-(<i>t</i> -BOC)-γ-L-glutamylaminoethyl)amino)naphthalene-1-sulfonic acid (γ-EDANS-α-(<i>t</i> -BOC)-L-glutamic acid)	100 mg
D-1537	4-dimethylaminoazobenzene-4'-sulfonyl chloride (dabsyl chloride)	100 mg
D-21	5-dimethylaminonaphthalene-1-sulfonyl chloride (dansyl chloride)	1 g
D-100	5-dimethylaminonaphthalene-1-sulfonyl hydrazine (dansyl hydrazine)	100 mg
D-2245	4-((4-(dimethylamino)phenyl)azo)benzoic acid, succinimidyl ester (dabcyl, SE)	100 mg
D-6216	ε-(4-((4-(dimethylamino)phenyl)azo)benzoyl)-α-9-fluorenylmethoxycarbonyl-L-lysine (ε-dabcyl-α-FMOC-L-lysine)	100 mg
F-11830	N ^ε -(9-fluorenylmethoxycarbonyl)-N ^ε -tetramethylrhodamine-(5-carbonyl)-L-lysine (α-FMOC-ε-TMR-L-lysine)	25 mg
F-11831	5-((2-(FMOC)-γ-L-glutamylaminoethyl)amino)naphthalene-1-sulfonic acid (γ-EDANS-α-FMOC-L-glutamic acid)	100 mg
Q-10464	QSY [®] 7 amine, hydrochloride	5 mg
Q-10193	QSY [®] 7 carboxylic acid, succinimidyl ester	5 mg
Q-21930	N ^ε -(QSY [®] 7)-N ^ε -(9-fluorenylmethoxycarbonyl)-L-lysine (α-FMOC-ε-QSY [®] 7-L-lysine)	5 mg
Q-10257	QSY [®] 7 maleimide	5 mg
Q-20131	QSY [®] 9 carboxylic acid, succinimidyl ester	5 mg
Q-20132	QSY [®] 21 carboxylic acid, succinimidyl ester	5 mg
Q-20133	QSY [®] 35 acetic acid, succinimidyl ester	5 mg
Q-21931	N ^β -(QSY [®] 35)-N ^ε -(9-fluorenylmethoxycarbonyl)-L-alanine (α-FMOC-β-QSY [®] 35-L-alanine)	5 mg
Q-20348	QSY [®] 35 iodoacetamide	5 mg
Q-20540	QSY [®] 35 methylamine	5 mg