Novabiochem®

innovations 2/05

SPPS of biotinylated peptides

Biotin-labeled peptides have many important applications in immunology and histochemistry, such as affinity purification [1], FRET-based flow cytometry [2], solid-phase immunoassays [3], and receptor localization [4].

The biotin label is most frequently located directly on the N-terminal group of the peptide, often without any regard to how this may affect peptide-target interactions, biotin-avidin binding, and the solubility properties of the resultant peptide. In many instances the products are poorly soluble, and have little biological activity and poor affinity for biotin. Problems can also arise during the synthesis of such N-terminally biotinylated peptides due to the poor solubility and reactivity of many of the reagents used for biotin introduction.

Novabiochem's biotin-loaded NovaTag™ resins provide a simple and elegant solution to these problems [5, 6]. Using these resins, biotinylated peptides are obtained directly following TFA cleavage, without the need for any additional biotinylation steps (Figure 1). Resins incorporate either an ethylenediamine or a 15 atom PEG spacer between the peptide and biotin to reduce steric hindrance.

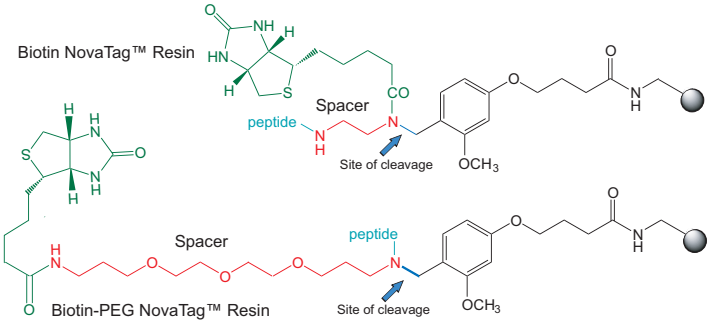

Fig. 1: Loaded Biotin NovaTag™ resins, showing point of attachment of peptide and site of cleavage.

The use of Biotin-PEG NovaTag™ resin is particularly advantageous because not only does the hydrophilic PEG chain confer better solubility to the peptide biotin conjugate, but its extended conformation leads to better avidin binding which can dramatically improve assay sensitivity as demonstrated in the Application 1. As the biotin is an integral part of the linker, its presence in every peptide chain is assured from the outset.

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Features and benefits of Biotin NovaTag™ resins

Features	Benefits
Biotin built into linker	No additional biotinylation step required
	Avoids use of poorly soluble biotinylation reagents
	Presence of biotin in every peptide chain assured
	Ideal for synthesis of large arrays of biotinylated peptides
Biotin introduced at C-terminus	N-terminal amino group free for introduction of other labels
	Peptides where binding site resides in N-terminus can be labeled without loss of activity
PEG spacer	Increases peptide solubility
	Less steric hindrance between peptide and biotin

Using Biotin NovaTag™ resins

Using Novabiochem's NovaTag™ resins for biotinylated peptide synthesis is extremely easy. Biotin NovaTag™ resin can be used directly in an automated synthesizer in the same manner as Rink amide resin. The Fmoc group is removed with 20% piperidine and the peptide assembled on the support using standard protocols. With Biotin-PEG NovaTag™, the procedure is the same except that the first residue should be coupled using HATU as described in Method 1, since this reaction involves acylation of a less reactive secondary amine. Cleavage from the resin can be effected using standard TFA cocktails, providing the C-terminally labeled biotinylated peptide.

The use of Biotin NovaTag™ and Biotin-PEG NovaTag™ resins is illustrated in Applications 1 & 2.

Method 1: Loading Biotin-PEG NovaTag™ resin

1. Suspend resin in DMF and leave to swell for 30 min.
2. Dissolve Fmoc-amino acid (2.5 eq.) and HATU (2.5 eq.) in minimum volume of DMF and add to resin. Add DIPEA (5 eq.) and mix.
3. The mixture is left to stand for 2 h with gentle agitation. A sample of resin can be removed and the loading determined using the Fmoc UV assay [7]. Repeat the coupling with fresh reagents if necessary.
4. The resin is removed by filtration, washed with DMF and used immediately in synthesis, or washed further with DCM and then MeOH, dried and stored for later use.

Peptide synthesis

Application 1: Synthesis of H-KKKKXXLLDXXXXXXXXXMKDEE-$NHCH_2CH_2$NH-biotin (23mer) [8]

Biotin NovaTag™ resin (354 mg, 0.145 mmole) was swollen in DMF and the Fmoc group removed with 20% piperidine. Peptide assembly was carried out on a Protein Technologies, Inc. Symphony peptide synthesizer using 30 min couplings of Fmoc-amino acids (3 eq.) activated with HCTU (3 eq.) in the presence of NMM (5 eq.). The biotinylated peptide was cleaved from the resin using Reagent K for 2.5 h. The crude peptide gave the HPLC profile shown in Figure 2. The minor component eluting ahead of the main product, is the corresponding methionine sulfoxide peptide.

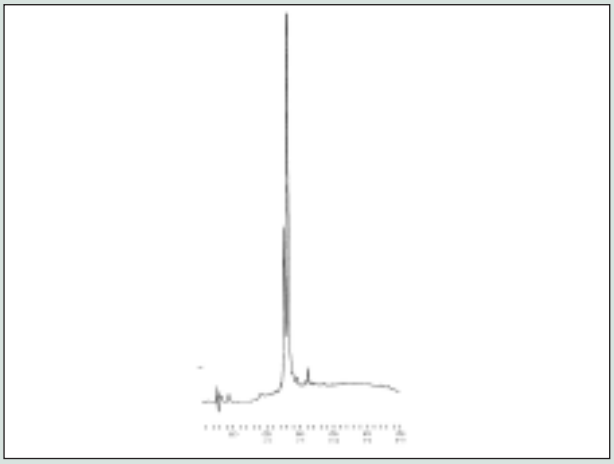

Fig. 2: HPLC elution profile of crude H-KKKKXXLLDXXXXXXXXXM-KDEE-$NHCH_2CH_2$NH-biotin prepared with Biotin NovaTag™ resin .

Application 2: Synthesis of H-KKKKXXLLDXXXXXXXXXMKDEE-NH-PEG-NH-biotin (23mer) [8]

Biotin-PEG NovaTag™ resin (345 mg, 0.145 mmole) was swollen in DMF and the Fmoc group removed with 20% piperidine. Peptide assembly was carried out on a Protein Technologies, Inc. Symphony peptide synthesizer using 30 min couplings of Fmoc-amino acids (3 eq.) activated with HCTU (3 eq.) in the presence of NMM (5 eq.). The biotinylated peptide was cleaved from the resin using Reagent K for 2.5 h. The crude peptide gave the HPLC profile shown in Figure 3. The minor component eluting ahead of the main product, is the corresponding methionine sulfoxide peptide.

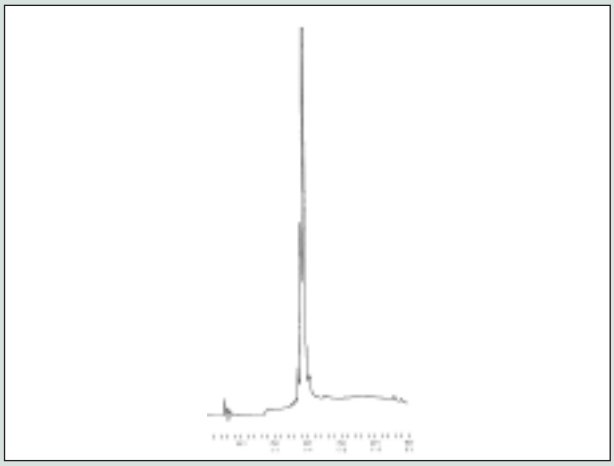

Fig. 3: HPLC elution profile of crude H-KKKKXXLLDXXXXXXXXXMK-DEE-NH-PEG-NH-biotin prepared with Biotin-PEG NovaTag™ resin.

Biotinylated-peptide design

When designing biotinylated peptides for use in assays, two of the most important considerations are the position of the biotin moiety and the nature of the spacer group between the peptide and biotin. This is because these can profoundly effect the strength of peptide-protein and biotin-avidin interactions and consequently the sensitivity of the assay. The importance of correct peptide presentation is illustrated in the following examples taken from developmental work on protein-binding and kinase assays carried out at Merck Pharma KGaA [9].

AlphaScreen™ protein-binding assay

The peptide-protein binding assay was conducted using the AlphaScreen™ technology as shown in Figure 4. N- and C-terminally biotin-labeled versions of the native peptide ligand immobilized on streptavidin-coated donor beads were screened against acceptor beads loaded with target protein. Only the peptide which was C-terminally labeled with PEG-biotin had acceptable solubility in the test buffer and showed significant levels of protein binding (Figure 5).

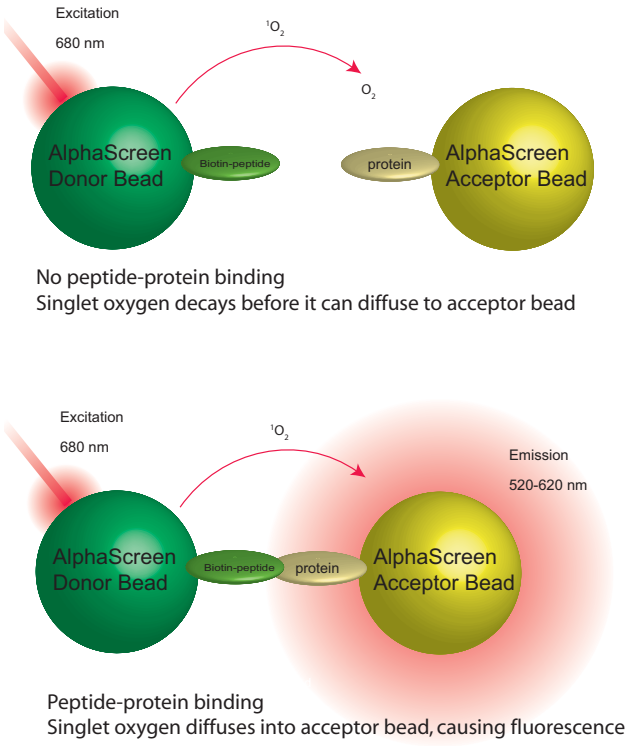

Fig. 4: Principles of the protein-peptide binding AlphaScreen™ assay.

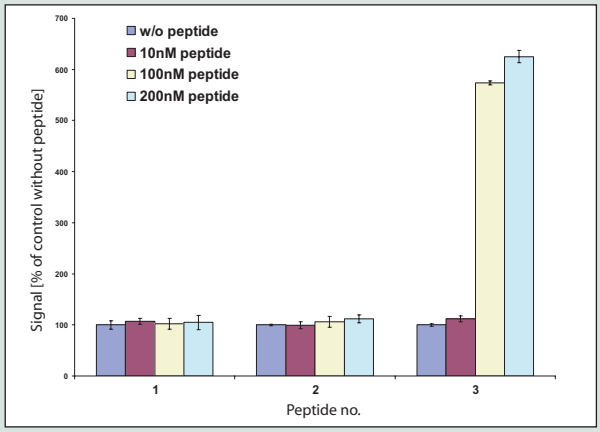

Fig. 5: AlphaScreen protein-binding assay. Peptide 1: N-biotin-XXXXX-NH_2; peptide 2: H-XXXXX-$NHCH_2CH_2NH$-biotin; peptide 3: H-XXXXX-NH-PEG-NH-biotin [8].

Kinase assay

N- and C-terminally biotin-labeled versions of a kinase substrate were evaluated in the assay shown in Figure 6. Peptides that were C-terminally labeled with biotin were found to give better reponses than those that were labeled on the N-terminus, whilst inclusion of a PEG spacer between the peptide and biotin appeared to have little effect (Figure 7).

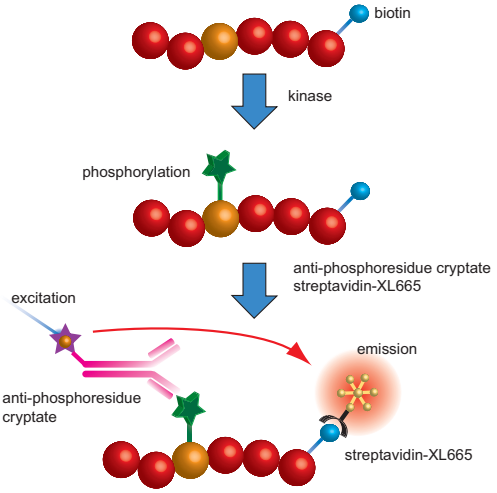

Fig. 6: Principles of the kinase assay.

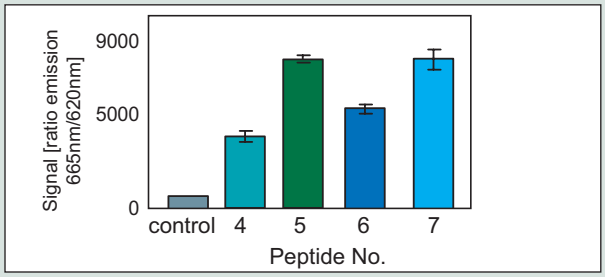

Fig. 7: Kinase assay. Peptide 4: biotin-KKKKXXLLDXXXXXXXXXMK-DEE-NH_2; peptide 5: biotin-NH-PEG-KKKKXXLLDXXXXXXXXXMK-DEE-NH_2; peptide 6: H-KKKKXXLLDXXXXXXXXXMKDEE-$NHCH_2CH_2NH$-biotin; peptide 7: H-KKKKXXLLDXXXXXXXXXMKDEE-NH-PEG-NH-biotin [8].

Ordering information

04-12-3901	**Biotin NovaTag™ resin**	500 mg 1 g
04-12-3908	**Biotin-PEG NovaTag™ resin**	500 mg 1 g

Novabiochem's other biotinylating reagents

01-63-0116	**Biotin-ONp**	1 g
01-63-0106	**Biotin-OSu**	1 g
01-63-0108	**2-Biotinyldimedone**	1 g 5 g
01-63-0133	**N-Biotinyl-NH-(PEG)$_2$-COOH·DIPEA (20 atoms)**	500 mg 1 g
04-12-1237	**Fmoc-Lys(biotin)-OH**	500 mg 1 g
04-12-1243	**Fmoc-Lys(biotinyl-ε-aminocaproyl)-OH**	500 mg 1 g
04-12-1250	**Fmoc-Glu(biotinyl-PEG)-OH**	500 mg 1 g

References

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7. M. Gude, et al. (2003) *Lett. Pept. Sci.*, **9**, 203.
8. Sequences proprietary.
9. O. Pöschke, unpublished results.

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