

# letters 3.12

# Novabiochem<sup>®</sup> NEW • NEW • NEW

- IMAC-based chemoselective purification tag
- Histidine derivative
- **Biotinylation reagents**

# NEW IMAC-based chemoselective tag for affinity purification of long peptides

**IMAC** Tag



## Features & Benefits

- Reversible TAG for purification of peptides using IMAC
- Orthogonal & complementary purification method to RP-HPLC
- Better recoveries than HPLC purification
- Better separation of closely related species than HPLC
- No optimization of separation required
- Easily automated as on-off process

RP-HPLC has a number of limitations for the purification of long synthetic peptides. The resolution is generally insufficient to effectively separate the target molecule from the mixture of closely related truncated and deletion products which arise during the synthetic process, and the recoveries of purified peptide are often very low. Furthermore, whilst the purified products may appear homogeneous by HPLC, they are often contaminated with co-eluting sequences which, because they are individually only present in small amounts, escape detection by mass spectrometry.

Novabiochem®'s new IMAC-based purification Tag provides an simple alternative to the use of RP-HPLC for the purification of long peptides. The method is extremely easy-to-use, gives higher recoveries than RP-HPLC, and because it has a selectivity orthogonal to RP-HPLC, it is more effective at removing closely eluting impurites. Furthermore, as the purification is an on-off process, it can be readily automated using standard HPLC or FPLC instrumentation.





Fig. 1: IMAC purification of peptides.

To use the tag, any failed couplings must be capped by treating the resin after each coupling step with  $Ac_2O$ . At the end of the synthesis, the resin is treated with the IMAC tag. Only the desired peptide sequence is labeled, as the only amino functionality available to react with the purification tag is the *N*-terminus of the full length peptide. The cleaved peptide is eluted through a Cu-loaded IDA column. Only the IMAC-labeled peptide is immobilized and the capped truncated peptides are washed away. The IMAC peptide is released from the column by elution with an acidic buffer. Temporary adjustment of the pH to 11 cleaves the tag. The final product can be desalted and given a polish by a quick pass through an RP-HPLC column [1].

Table 1 compares results obtained for some peptide purifications carried out by RP-HPLC and IMAC. IMAC purification was found to give consistently higher recoveries and purities compared to RP-HPLC [1].

Table 1: RP-HPLC vs IMAC purification.

Peptide	RP-HPLC		IM/	IMAC	
	Recovery (%)	Purity (%)	Recovery (%)	Purity (%)	
YLLPRRGPRLGV	21	95	38	99	
Gly-GLP (2-36)	5	81	22	89	
Ubiquitin	21	82	62	89	

Cat.No.	Product	Contents	Price EUR
851208	IMAC tag	250 mg	85.00
NEW	Sold for research use only.	1 g	325.00
	Other tags		
851092	4-Nitrophenyl 2- (octadecylsulfonyl)ethyl	250 mg	52.00
	carbonate	1 g	180.00

# NEW Derivative for synthesis of histidinecontaining protected fragments

#### Fmoc-His(Clt)-OH



## Features & Benefits

- Ideal tool for the synthesis of His-containing protected peptides
- No loss of Clt group during HFIP/DCM or 1% TFA in DCM treatment
- Gives cleaner protected peptide fragments than those obtained using Fmoc-His(Trt)-OH

The use of Fmoc-His(Trt)-OH for the production of protected peptide fragments can result in the formation of a mixture of fully and partially protected peptides because loss of the *im*-trityl group can occur during the HFIP/DCM or 1% TFA in DCM treatments used to release products from 2-chlorotrityl or HMPB-AM resins. It is for this reason that the Novabiochem® brand has introduced Fmoc-His(Clt)-OH. The *im*-Clt group is completely stable to the mild acids used to cleave protected fragments but is removed with 95% TFA, enabling the preparation of protected peptides free from by-products containing unprotected His residues.

Cat.No.	Product	Contents	Price EUR
852371	Fmoc-His(Clt)-OH	5 g	100.00
NEW		25 g	400.00

# **PAL linker**

Fmoc-PAL-linker



Fmoc-PAL-linker is an Fmoc-protected version of Barany's aminomethyldimethoxyphenoxyvaleric acid linker [2]. It can be attached to any suitable aminofunctionalized resin to produce a support for the synthesis of peptide amides by Fmoc SPPS. Studies have shown the acid sensitivity of this linker to be around twice that of the Rink amide linker [3].

Cat.No.	Product	Contents	Price EUR
855140	Fmoc-PAL-linker	1 g	40.00
NEW		5 g	160.00

# **Biotinylation reagents**

NEW • Resin for synthesis of C-terminal Lys(biotinyl-εaminocaproyl) peptides

### $\mathsf{Fmoc-Lys}(\mathsf{biotinyl-}\epsilon\text{-}\mathsf{aminocaproyl})\text{-}\mathsf{NovaSyn}^{\circledast}\,\mathsf{TG}^R\,\mathsf{HMP}\,\mathsf{resin}$



#### **Features & Benefits**

- Eliminates problems with loading resin with poorly soluble Fmoc-Lys(biotinyl-eaminocaproyl)-OH
- Ideal for synthesis of long peptides as the base resin is NovaSyn<sup>®</sup> TG<sup>R</sup>
- TFA cleavage provides C-terminally biotinylated peptide

Biotin-labeled peptides have many important applications in immunology and histochemistry that exploit the high affinity of avidin and streptavidin for biotin.

The Novabiochem<sup>®</sup> brand has one of the broadest product ranges for the synthesis of biotinylated peptides by solid phase synthesis. Tools are available for inclusion of biotin at both *N*- and *C*-termini of the peptide, as well as on the side-chain of amino acids within the peptide chain.

Fmoc-Lys(biotinyl- $\varepsilon$ -aminocaproyl)-NovaSyn® TG<sup>R</sup> HMP resin is the latest addition to our products for introduction of biotin at the C-terminus of peptides. The lysine is attached to the resin *via* an acid-cleavable HMP linker, which on treatment with TFA releases the corresponding peptide acid. The base resin is NovaSyn TG<sup>R</sup>, which is a novel PEG-polystyrene support designed specifically for the synthesis of long and difficult peptides.

#### Biotin-PEG NovaTag<sup>™</sup> resin

#### Fmoc–PEG Biotin NovaTaq<sup>™</sup> resin



Novabiochem<sup>®</sup> also offers Biotin-PEG NovaTag<sup>™</sup> resin and Fmoc-PEG Biotin NovaTag<sup>™</sup> resins for the *C*-terminally biotinylated peptides [4]. Using these resins, biotinylated peptides are obtained directly following TFA cleavage incorporating a 15 atom PEG spacer between the *C*-terminal carboxyl group of the peptide and biotin to reduce steric hindrance. The hydrophilic PEG chain not only confers better solubility to the peptide biotin conjugate but its extended conformation leads to better avidin binding with consequently dramatically improved assay sensitivity [5]. Fmoc-Lys(biotinyl-ε-aminocaproyl)-NovaSyn® TG<sup>R</sup> HMP and Fmoc-PEG Biotin NovaTag<sup>™</sup> 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<sup>™</sup> resin, the procedure is the same except that the first residue should be coupled using HATU, 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.

#### N-terminal and side-chain biotinylation

Biotin-ONp









For coupling of biotin to amines on the solid phase the use of Biotin-ONp is strongly recommended [6]. The solubility of this reagent in DMF or NMP is much greater than that of Biotin-OSu, and it couples with amines much more rapidly: typically in 40 min as opposed to 12 h for Biotin-OSu (Figure 2). The Novabiochem brand also offers N-Biotinyl-NH-PEG<sub>2</sub>-COOH and N-Biotinyl-NH-PEG<sub>11</sub>-COOH, which incorporate of PEG chain between the peptide and biotin moiety. These derivatives have good solubility in DMF and can be coupled using standard methods.





Fmoc-Asp(biotinyl-PEG)-OH

Fmoc-Glu(biotinyl-PEG)-OH



Alternatively, preformed derivatives such as Fmoc-Lys(biotin)-OH, Fmoc-Lys(biotinyl-ɛ-aminocaproyl)-OH, Fmoc-Asp(biotinyl-PEG)-OH, and Fmoc-Glu(biotinyl-PEG)-OH can be used to introduce biotin at a precise location within a peptide chain. The use of Fmoc-Asp(biotinyl-PEG)-OH or Fmoc-Glu(biotinyl-PEG)-OH is particularly recommended as they possess good solubility in DMF (Fmoc-Glu(biotinyl-PEG)-OH, 0.5 mmole/ml; Fmoc-Lys(biotin)-OH, <0.05 mmole/ml; Fmoc-Lys(biotinyl-ɛ-aminocaproyl)-OH, <0.05 mmole/ml) and offers the same benefits as the other previously PEGylated biotin derivatives in terms of enhancing peptide solubility and bioactivity.

Cat.No.	Product	Contents	Price EUR
856193	Fmoc-Lys(biotinyl-ε-aminocaproyl)-	250 mg	250.00
NEW	NovaSyn® TG <sup>R</sup> HMP resin	1 g	475.00
851209	Biotin	1 g	30.00
NEW		5 g	120.00
		25 g	480.00
855055	Biotin-PEG NovaTag™ resin	500 mg	260.00
		1 g	500.00
855145	Fmoc-PEG Biotin NovaTag <sup>™</sup> resin	500 mg	285.00
		1 g	550.00

851027	Biotin-ONp	1 g	160.00
851029	N-Biotinyl-NH-PEG <sub>2</sub> -COOH $\cdot$ DIPEA	500 mg	258.00
		1 g	426.00
852340	N-Biotinyl-NH-PEG <sub>11</sub> -COOH	250 mg	320.00
		1 g	960.00
852113	Fmoc-Asp(biotinyI-PEG)-OH	500 mg	285.00
		1 g	510.00
852102	Fmoc-Glu(biotinyl-PEG)-OH	500 mg	285.00
		1 g	510.00
852097	Fmoc-Lys(biotin)-OH	500 mg	195.00
		1 g	380.00
852100	Fmoc-Lys(biotinyl-ɛ-aminocaproyl)-OH	500 mg	165.00
		1 g	295.00

#### References

1. Novabiochem Innovations 03/2012.

2. F. Albericio & G. Barany (1987) Int. J. Peptide Protein Res., 30, 206

3. M. S. Bernatowicz, et al. (1989) Tetrahedron Lett., 30, 4645.

4. J. Beythien & P. White (2003) Biopolymers, 71, 362

5. Novabiochem Innovations 03/2005.

6. B. Baumeister, et al. (2005) Int. J. Pept. Res. Ther., 11, 139.

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