



Developer Kit 25

Manual

Catalog number: 180484002

(reductively cleavable PEC-Linker RC+)

V2.1 - 08.05.2020

Introduction

Belyntic's Peptide Easy Clean (PEC) technology breaks barriers in peptide purification.

The key for a universal application is our novel purification linker, the PEC-Linker.

The target peptide binds on our activated filter material via robust oxime ligation. Our innovative chemistry allows the traceless release of the purified peptide without side reactions.

This manual provides step-by-step instructions for the PEC purification using our kits, alongside with helpful information and notes to assure a successful use.

Please watch our product video on YouTube (<https://youtu.be/gihpfHr1bfl>) for step-by-step visual guidance. Please also refer to the FAQ on our website [belyntic.com](https://www.belyntic.com).

Now, let's get ready to re-think peptide purification!



Contents

Product Description	5
<i>Kit Content</i>	5
<i>About this manual</i>	5
<i>Safety Information</i>	5
<i>Storage Conditions</i>	5
<i>Product Use</i>	5
Preparations	6
<i>Other Chemicals & Consumables</i>	7
<i>Preparation of Buffers</i>	8
Step 1: PEC-Linker Coupling	9
Step 2: TFA Cleavage	10
Step 3: Dissolution & Bead Conditioning	11
Step 4: Immobilization (Catch)	12
Step 5: Washing	12
Step 6: Cleavage & Work-up (Release)	13
Appendix	14
<i>Reaction overview</i>	14
<i>Belyntic's PEC Delta Masses</i>	15
<i>Abbreviations</i>	16
<i>Your Notes</i>	18

Product Description

Kit Content

quantity	label / description	amount
1	Activated filter material / Agarose100	250 mL
1	PEC-Linker / RC+	25 g

About this manual

This manual is written for exemplary peptide purification in 100 μ mol synthetic scale. If you run other scales, adjust all given quantities accordingly (e.g. for 200 μ mol scale use every amount two-fold, 1 g of DTT instead of 0.5 g etc.). Before dispensing into suitable fritted cartridges, shake agarose container heavily to ensure equal distribution of beads.

Safety Information

Please read the Safety Data Sheets, available at belyntic.com before use.

Storage Conditions

Upon receipt, store the kit components in the fridge at 2-8°C. The agarose beads should not be frozen or dried under reduced pressure. Buffers (cf. page 8) can be stored for 2 weeks in the fridge at 2-8°C.

Product Use

For Research Use Only. The use in or for clinical or diagnostic products, applications, or services is excluded.

Preparations

Purification Considerations

- PEC-Linker RC+ will be installed at the *N*-terminus of the peptide
- reductive conditions will be applied in the purification process
- azide and disulfide groups in the peptide might be reduced

Precautions During Peptide Synthesis

- **acetylation after each amino acid coupling (capping) step is crucial to ensure a successful PEC purification**
efficient conditions for blocking unreacted amino groups are 2 M acetic anhydride (20 vol%) with either 2 M pyridine (18 vol%) or 2 M 2,6-lutidine (21 vol%) in DMF for 5 min¹
- **pay special attention to possible side reactions during SPPS**
 - minimize by-products within the full-length peptide (e.g. aspartimide)
 - minimize by-products during TFA cleavage,¹ ideally use Fmoc-Lys(Trt)-OH to prevent *t*Bu attachment on Lysine² and Fmoc-Cys(StBu)-OH to prevent alkylation on -SH during TFA cleavage
 - use Rink Amide (RAM) or Ramage resins for C-terminal peptide amides and avoid Wang-linker for C-terminal peptide acids
 - avoid amino acid cross-contamination
 - avoid incomplete Fmoc-deprotection, e.g., at Ala-, Ile-, Leu-rich sequences
- **avoid aldehydes and/or ketones in ether and solvents**
the free oxyamine on PEC-linker RC+ will readily react with traces and contaminations of ketones and/or aldehydes that will deactivate it

¹ O. Reimann, O. Seitz, D. Sarma, R. Zitterbart, *J Pept Sci* **2019**, 25, e3136.

² J. Pawlas, U. Törnvall, *Proceedings of the 33rd European Peptide Symposium* **2014**, 108-109.

Other Chemicals & Consumables

Reagents

- ethyl-2-cyano-2-(hydroxyimino) acetate (Oxyma) or 1-hydroxybenzotriazol (HOBT) as coupling reagent
- scavengers for TFA cleavage cocktail, e.g. TIS, EDT, thioanisole, phenol
- guanidinium chloride (GdmCl) – in case of technical grade, filter the solution prior to use
- sodium chloride (NaCl)
- L-cysteine (CAS: 52-90-4, no hydrochloride)
- dithiothreitol (DTT), Dithioerythritol (DTE) works as well but is odorous

Acids / Bases

- *N,N*-diisopropylethylamine (DIEA)
- trifluoroacetic acid (TFA)
- sodium hydrogen carbonate (NaHCO₃)
- citric acid (anhydrous or monohydrate)
- sodium carbonate (Na₂CO₃, anhydrous)

Solvents

- *N,N*-dimethylformamide (DMF), ≥ 99.5% (peptide synthesis grade)
- dimethyl sulfoxide (DMSO), ≥ 99.5% (synthesis grade)
- dichloromethylene (CH₂Cl₂), ≥ 99.5% (synthesis grade)
- acetonitrile (MeCN), ≥ 99.5% (synthesis grade)
- deionized water
- ethanol (EtOH), denatured
- suitable ether for peptide precipitation

Consumables

- fritted cartridges to hold the agarose beads (e.g. 20 mL for 100 μmol)



Preparation of Buffers

□ **Buffer#1**

dissolve 19.2 g Citric Acid (anhydrous, or 21.0 g of monohydrate) together with 8.75 g Na_2CO_3 (anhydrous) slowly in 1 L water.

□ **Buffer#2 (7 M GdmCl in Buffer#1)**

dissolve 13.4 g of GdmCl in 20 mL Buffer#1

□ **Wash A: DMSO with 0.9 M GdmCl**

dissolve 13 g of GdmCl in 150 mL of DMSO

□ **Wash B: EtOH/H₂O (7:3) with 0.1 M NaCl**

dissolve 1168 mg of NaCl in 60 mL water **first**, then add 140 mL EtOH

□ **NaHCO₃ pH 8:**

dissolve 2.5 g of NaHCO₃ in 50 mL water to give a pH of 8

Equipment

- shaking device for fritted cartridges
- centrifuge (recommended) or filtration apparatus to collect peptide precipitates
- vacuum manifold or vacuum flask for parallel setup (optional)

Kit Handling

- process single purifications by hand, using the plunger and push-pull movements
- for parallel setups, use a vacuum manifold or a vacuum flask with a septum

Step 1: PEC-Linker Coupling

- swell resin in DMF for 15 min and remove before PEC-Linker addition
- weigh in the amount of PEC-Linker and Oxyma or HOBt (Table 1) and combine

Table 1: Amounts for PEC-Linker RC+ coupling per 100 μmol peptide

PEC-Linker RC+ (4 eq.)	301 mg	400 μmol
Oxyma/HOBt (6 eq.)	86/81 mg	600 μmol
add DMF to have ~ 1.3 mL	1000 μL	0.3 M PEC-Linker RC+
DIEA (6 eq.)	105 μL	600 μmol

- add DMF to dissolve PEC-Linker RC+ and Oxyma or HOBt to have a 0.3 M solution, then add DIEA (Table 1)
- quickly add the yellow solution to synthesis resin

Note: Other common solvents such as CH_2Cl_2 , DMSO, or *N*-methylpyrrolidone (NMP) can be used.

- shake for ≥ 2 hours (overnight is possible)
- wash synthesis resin with $3x \geq 2$ mL DMF and $3x \geq 2$ mL CH_2Cl_2
- dry synthesis resin before TFA cleavage

Note: On an automated synthesizer, add the pre-mixed solution of the PEC-Linker with Oxyma (or HOBt) to the peptide and then add the base directly to the coupling mixture. *N*-methylmorpholine may be used instead of DIEA.

Note: You may pause here and store the linker-modified peptide on synthesis resin below 8 $^\circ\text{C}$.

Step 2: TFA Cleavage

- choose and prepare a proper cleavage cocktail for your peptide (≥ 10 mL/100 μ mol synthesis scale)

By-products caused by re-addition of protecting groups cannot be removed with PEC, please use proper scavengers: Reagent Bel TFA/H₂O/PhSMe/EDT/TIS (83:5:5:5:2) or a non-odorous cocktail of TFA/H₂O/DTT/TIS (88:4:6:2) are recommended.

- add cleavage cocktail to synthesis resin and carry out cleavage for 2 hours; only exceed cleavage time in case your peptide sequence requires longer cleavage times (ideally use PhSMe for Arg-rich sequences)
- if you want to purify methionine containing sequences, please use Reagent Bel and add 140 μ L TMSBr after 105 min to the cleavage cocktail and shake for 15 min
- precipitate the peptide in cold ether (e.g., Et₂O, *i*Pr₂O, MTBE)

Aldehyde- and ketone-free ether is crucial during this step; in doubt use THF/heptane (1:1) as an alternative.

- collect the precipitate and wash with ether

Note: You may pause here and store the crude linker-modified peptide as a dried solid below 8 °C.

Check the delta mass list in Table 2 (cf. page 15) for crude analysis. On some analytical LC systems, the linker-modified peptide cannot be detected or shows lower purities. Dilute your sample with a 50 mg/mL solution of methoxyamine hydrochloride (CAS: 593-56-6) to prevent analytical artifacts due to aldehydes or ketones. However, if you still do not see linker-modified peptide, please continue the purification process described in this manual as the desired peptide will be visible again after PEC-Linker cleavage.

Step 3: Dissolution & Bead Conditioning

- take a 20 mL cartridge and fill it with 3 mL agarose beads suspension and remove supernatant by using a vacuum manifold or by push-pull movements
- wash agarose beads 3x with 5 mL water
- wash agarose beads 3x with 5 mL Buffer#1
- wash agarose beads once with 5 mL DMSO
- close the cartridge at the bottom and add 1.5 mL DMSO to the agarose beads
- dissolve the peptide in 4.5 mL DMSO and make sure that it is fully dissolved
- slowly add 500 μ L Buffer#2 (Buffer#1 with 7 M GdmCl, see page 8)

Note: Other solvents, such as DMF or MeCN up to 90 vol% in Buffer#1 or pure Buffer#1 may be used. Ketone- or aldehyde-containing solvents are not tolerated. For very hydrophobic peptides, pure HFIP may be used. Make sure that the pure HFIP is trifluoroacetone-free (procedure below), since it will react with the PEC-Linker and reduce yield.

1,1,1-trifluoroacetone removal from HFIP: Weigh 1% (w/v) cysteine for the corresponding amount of HFIP (e.g. 1 g of cysteine for 100 mL of HFIP) and reflux at 58°C for one hour. Afterwards, gain the purified HFIP by distillation on a rotary evaporator and make sure that no ketone (e.g. acetone) has been used on the rotary evaporator prior to distillation.

Step 4: Immobilization (Catch)

- add the dissolved peptides
- close the top of the cartridge with a plunger and immobilize peptides by shaking for 90 min

Please make sure to disperse the agarose beads well while shaking; if you observe pellet-formation in the cartridge, break it apart.

Note: You may monitor immobilization efficiency by LC or MS analysis of the supernatant prior to addition of L-cysteine.

- prepare Blocking Buffer: prepare 2 w% solution of L-cysteine by dissolving 100 mg L-cysteine (CAS: 52-90-4, not the hydrochloride since you have to adjust pH) and dissolve in 5 mL Buffer#1 per peptide
- remove the immobilization mixture from the agarose beads and wash 3x with 5 mL DMSO
- add 5 mL of Blocking Buffer to each cartridge and shake for 15 min to block unreacted aldehyde groups and to reverse imines to amines

Step 5: Washing

Each wash is 3x 5 mL per cartridge

- **wash A:** DMSO with 0.9 M GdmCl
- **wash B:** EtOH/H₂O (7:3) with 0.1 M NaCl
- remove supernatant from the agarose beads

Note: You may pause here by washing once with water and then store the immobilized peptide on the agarose beads in the fridge at 2-8°C.

Step 6: Cleavage & Work-up (Release)

- prepare Reducing Agent: dissolve 500 mg DTT in 5 mL 5 w% aq. NaHCO₃ (0.6 M; pH 8) per peptide
- add 5 mL MeCN to each cartridge followed by 5 mL of the Reducing Agent and shake for 15 min; shake for 60 min when Cys (StBu) has been used
- wash 3x with 5 mL water, 2x with 5 mL MeCN and discard to remove excess of DTT

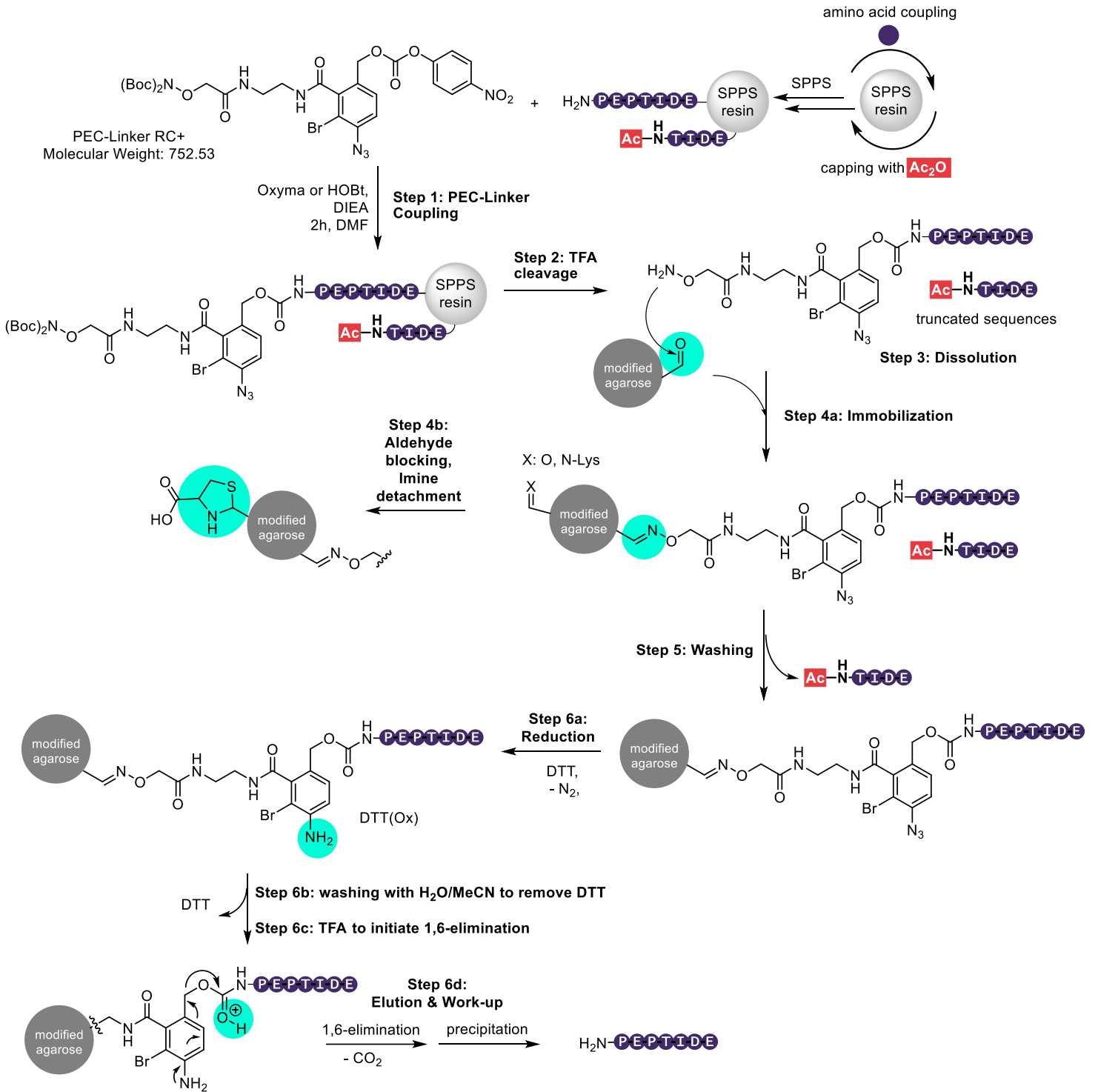
From this point on, collect all filtrates in one beaker or centrifuge tube since they hold the product.

- add 2 mL TFA/water (40:60) and shake for 60 min to release the peptide
- add 2 mL TFA to the cartridge and collect the solution in a centrifuge tube or beaker **without ether inside**
- elute 2x with 2 mL TFA/water (95:5) each into the same tube or beaker
- precipitate the peptide **by adding** ≥ 40 mL of **cold ether** (e.g., Et₂O, iPr₂O, MTBE, THF/heptane 1:1) **to the TFA/water mixture** that contains the peptide
- collect the precipitate by centrifugation or filtration and wash it once with ether
- dissolve the peptide and lyophilize (if required)

Note: Sometimes double peaks with identical masses are observed. After lyophilization, they usually converge to a single peak. If you don't want to lyophilize, dry the ether precipitate under reduced pressure prior to peptide dissolution.

Appendix

Reaction overview



Belyntic's PEC Delta Masses

Table 2: Relevant delta masses during the purification process

<p>+412.03 Da / 414.03 Da (MH²⁺: +206.52 m/z, MH³⁺: +137.67 m/z)</p>	<p>PEC-Linker RC+ on peptide</p>
<p>+40.05 Da (MH²⁺: +20.03 m/z, MH³⁺: +13.33 m/z)</p>	<p>acetone oxime of peptide-linker conjugate, +453.08 Da with PEC-Linker RC+</p>
<p>+26.03 Da (MH²⁺: +13.02 m/z, MH³⁺: +8.68 m/z)</p>	<p>acetaldehyde oxime of peptide-linker conjugate, +439.06 Da with PEC-Linker RC+</p>
<p>+12.01 Da (MH²⁺: +6.01 m/z, MH³⁺: +4.00 m/z)</p>	<p>formaldehyde oxime of peptide-linker conjugate, +425.04 Da with PEC-Linker RC+</p>
<p>+56.07 Da (MH²⁺: +28.04 m/z, MH³⁺: +18.69 m/z)</p>	<p>reattached tBu; thiols (e.g. EDT) should be used in the TFA cleavage cocktail (make sure they are not oxidized)</p>
<p>+106.05 Da (MH²⁺: +53.03 m/z, MH³⁺: +35.35 m/z)</p>	<p>para-hydroxybenzyl cation, generated during TFA cleavage; use RAM-linker or add EDT and 1,3-Dimethoxybenzene during cleavage</p>
<p>+252.06 Da (MH²⁺: +126.03 m/z, MH³⁺: +84.02 m/z)</p>	<p>non-cleaved or reattached Pbf; treat purified peptide with Reagent Bel or another thioanisole-containing TFA cocktail such as Reagent K for 3-4 h.</p>



Abbreviations

AcOH	acetic acid
Buffer#1	immobilization buffer
Buffer#2	7 M guanidinium chloride in immobilization buffer
CAS	Chemical Abstracts Service
DIEA	<i>N,N</i> -Diisopropylethylamine
DMF	<i>N,N</i> -Dimethylformamide
DTE	dithioerythritol
DTT	dithiothreitol
EDT	ethanedithiol
Et ₂ O	diethyl ether
EtOH	ethanol
GdmCl	guanidinium chloride
HFIP	1,1,1,3,3,3-Hexafluoro-2-propanol
HOBt	1-hydroxybenzotriazole
<i>i</i> Pr ₂ O	diisopropyl ether
LC	liquid chromatography
MeCN	acetonitrile
MS	mass spectrometry
MTBE	<i>t</i> -butyl methyl ether
Oxyma	ethyl-2-cyano-2-(hydroxyimino) acetate
Pbf	pentamethyldihydrobenzofuran-5-sulfonyl
PEC	Peptide Easy Clean
PhSMe	thioanisole
RAM	Rink Amide AM resin; 4-(2,4'-Dimethoxyphenyl-Fmoc-aminomethyl)-phenoxyacetamido-aminomethyl resin
SDS	safety data sheet
SPPS	solid phase peptide synthesis
<i>t</i> Bu	<i>tert</i> -butyl
TFA	trifluoro acetic acid
THF	tetrahydrofuran
TIS	triisopropylsilane
TMSBr	trimethylsilyl bromide



Technical Support

Please watch our product video on YouTube for step-by-step visual guidance (<https://youtu.be/gihpfHr1bfl>). Please also refer to the FAQ on our website belyntic.com.

For further technical support, submit your questions directly via email to support@belyntic.com or call +49 30 8104-1113.

Safety Data Sheets (SDS)

Safety Data Sheets (SDS) are available on our website belyntic.com.

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Your Notes



Your Notes



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