



High-Throughput Kit 48x 20 μ mol

Manual

Catalog number: 180484102

(reductively cleavable PEC-Linker RC+)

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Introduction

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

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

The target peptides are chemically retained on our activated filter material via robust oxime ligation. Our innovative chemistry allows traceless release of the purified peptides.

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!



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Product Description

Kit Content

quantity	label / description	amount
1	PEC filter plate with activated filter material / 50% suspension of Agarose100 provided in 48-well filter plate 800 µL/well, closed with top- and bottom-sealing-mat	38.4 mL
1	PEC-Linker / RC+	3.2 g
1	Buffer salt / mixture of citric acid-sodium carbonate	5.6 g
1	Blocking agent / L-cysteine	1.0 g
1	Reducing agent / Dithiothreitol	5.0 g
1	Peptide collection plate / perforated deep-well plate to collect purified peptides and perform freeze-drying in the plate	

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 key 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

- **special attention must be paid to possible side reactions during SPPS**
 - minimize by-products within the fulllength chain (e.g. aspartimide)
 - minimize by-products during TFA cleavage¹, ideally use Fmoc-Lys(Trt)-OH to prevent *t*Bu on Lysine² and Fmoc-Cys(StBu)-OH to prevent alkylation on -SH during TFA cleavage
 - use Ramage- or Sieber-amide resins for C-terminal peptide amides and chlorotrityl resin for C-terminal peptide acids (avoid Wang-linker)
 - N-terminal glutamine bearing sequences may show 10-20% of pyroglutamate after purification, contact us for support if you want to purify N-terminal glutamine peptides

- **avoid aldehydes and/or ketones in ether and solvents**

the free oxy-amine on PEC-linker 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, thioanisol, phenol
- guanidinium chloride (GdmCl) - in case of technical grade, filter the solution prior to use
- sodium chloride (NaCl)

Acids / Bases

- *N,N*-diisopropylethylamine (DIEA)
- trifluoroacetic acid (TFA)
- sodium hydrogen carbonate (NaHCO₃)

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 (Et₂O and n-heptane 3:1), ≥ 99.5% (synthesis grade, acetone-free)
- 1,1,1,3,3,3-hexafluoro-propan-2-ol (HFIP), if using synthesis grade (≤99%) please reflux 1 h with 1 w% L-cysteine to remove trifluoroacetone, distill then to get pure HFIP



Preparation of Buffers

□ **Buffer#1**

dissolve 5.6 g of the supplied buffer salt slowly in 200 mL water (final pH = 4.5, caution: strong CO₂ evolution); To prepare more Buffer#1 see below³

□ **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 & Processing

please don't hesitate to contact us, if in doubt about suitable equipment

- shaking device for deep-well plates, ideally with 800 rpm
- centrifuge with rotor for deep-well plates
- Belyntic's Acrylic Vacuum Manifold, catalog number: 180484150. Other manifolds cannot be recommended since they may produce cross-contaminations
- 30-300 µL multichannel pipette (8- or 12-channel) or dispenser with 8- or 12-channel manifold exit

³Buffer#1: dissolve 19.2 g Citric Acid (anhydrous, or 21.0 g of monohydrate) together with 8.75 g Na₂CO₃ (anhydrous) slowly in 1 L water.

Step 1: PEC-Linker Coupling

Note: The following procedure describes manual coupling of PEC-Linker RC+ after peptide assembly. The PEC-Linker can also be coupled to the peptides on a synthesizer; higher volumes might be necessary due to synthesizer dead volume. The base (DIEA or *N*-methylmorpholine) must be added to the pre-mixed solution of the PEC-Linker with Oxyma (or HOBt) before coupling.

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

Table 1: PEC-Linker coupling for 48x 20 μ mol peptide

PEC-Linker RC+ (>4 eq.)	3200 mg	4255 μ mol
Oxyma/ HOBt (>6 eq.)	907/862 mg	6383 μ mol
add DMF to have ~13.8 mL	10630 μ L	0.3 M PEC-Linker
DIEA (>6 eq.)	1116 μ L	6383 μ mol

- add DMF to dissolve PEC-Linker and Oxyma or HOBt (Table 1)
- then add DIEA (Table 1, on a synthesizer DIEA or *N*-methylmorpholine should be added directly in a pre-activation vessel before coupling)
- quickly add 300 μ L of the yellow solution to each peptide

Note: Other common solvents can be used (e.g. CH_2Cl_2 , DMSO or NMP).

- couple for ≥ 2 h while shaking
- wash synthesis resin with 3x ≥ 500 μ L DMF and 3x ≥ 500 μ L CH_2Cl_2

Note: You may pause here and store the linker-modified peptide on synthesis resin below 8°C.

Step 2: TFA Cleavage

- choose and prepare proper cleavage cocktail for your peptides (2 mL/peptide = 96 mL)

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 peptide resin and carry out cleavage for 2 h, only exceed cleavage time in case your peptide sequences require longer cleavage times (ideally use PhSMe for Arg-rich sequences)
- for methionine containing sequences, please use Reagent Bel, add 28 µL TMSBr after 105 min to the cleavage cocktail and shake for 15 min
- precipitate the peptides in cold ether (e.g. Et₂O, iPr₂O)

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

- collect the precipitates and wash with ether

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

If crude analysis is performed, check delta mass list in Table 2 (cf. page 15). 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), that will prevent analytical artifacts due to aldehydes or ketones. However, if you still don't 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

- dissolve the peptides in 900 μ L DMSO each and make sure that they are fully dissolved

Note: For very hydrophobic peptides HFIP can be used (make sure it is free of trifluoroacetone, solvent section page 7). Other solvents, such as DMF or MeCN up to 90 vol% in Buffer#1, may be used. Ketone- or aldehyde-containing solvents are not tolerated.

- slowly add 100 μ L Buffer#2 (Buffer#1 with 7 M GdmCl, see page 8); **don't add Buffer#2 when using HFIP as peptide solvent**
- take the PEC filter plate that is prefilled with agarose suspension provided in your High-Throughput Kit, remove top- and bottom-sealing-mat, place it in the vacuum manifold and remove the supernatants
- wash 3x 600 μ L/well with water and 3x 600 μ L/well with Buffer#1 (2.4 mL/4-wells, 3.6 mL/6-wells, 90 mL in total each)
- wash once with 600 μ L/well DMSO **or again with 600 μ L/well water when using HFIP as peptide solvent**
- close the PEC filter plate with the bottom-sealing-mat and add 400 μ L/well of the solvent used for peptide dissolution

Step 4: Immobilization (Catch)

- add the dissolved peptides to their respective wells
- apply the top-sealing-mat and immobilize peptides by shaking for ≥ 4 h (overnight possible, when not dealing with methionine containing sequences)

Please make sure to use a shaker that is suitable for deep-well plates and use a shaking speed of 800 rpm.

Before removing sealing-mats, centrifuge the plates for 1 min at ≥ 4000 rpm to prevent cross-contaminations.

If you have methionine containing peptides and you want to use DMSO, please use DMSO purged with inert gas (N_2 or Ar) and try not to exceed immobilization time of 4 h.

- remove the sealing-mats from the PEC filter plate and drain the immobilization supernatants by usage of the vacuum manifold
- wash 3x 600 μ L/well with DMSO (2.4 mL/4-wells, 3.6 mL/6-wells)

Note: You may monitor immobilization efficiency by LC or MS analysis of the supernatant of single wells prior to addition of Blocking Buffer.

- prepare Blocking Buffer (2 w% solution of L-cysteine): dissolve all L-cysteine (1 g) provided in your High-Throughput Kit in 50 mL Buffer#1
- close the PEC filter plate with the bottom-sealing-mat and add 1 mL/well of Blocking Buffer
- apply top-sealing-mat and shake for 30 min to block unreacted aldehyde groups and to liberate imines
- remove the sealing-mats, place the PEC filter plate on the vacuum manifold and remove the Blocking Buffer

Step 5: Washing

Each wash is 3x 600 μ L/well (2.4 mL/4-wells, 3.6 mL/6-wells, 90 ml in total)

- **wash A:** DMSO with 0.9 M GdmCl
- **wash B:** EtOH/H₂O (7:3) with 0.1 M NaCl

Note: You may pause here by storing the immobilized peptides after one additional H₂O wash in the fridge at 2-8 °C.

Step 6: Cleavage & Work-up (Release)

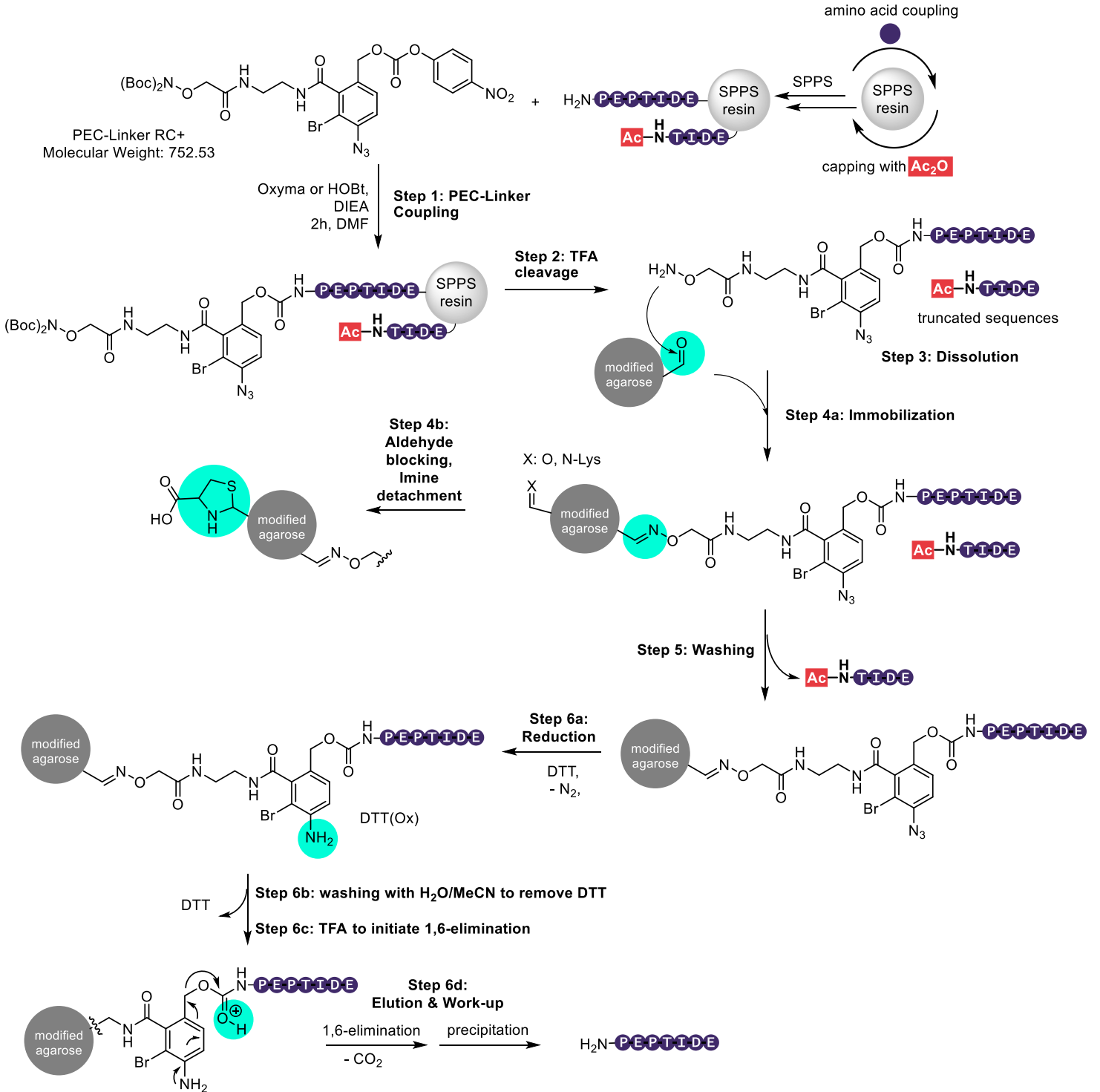
- prepare Reducing Agent: dissolve 5.0 g DTT in 50 mL 5 w% aq. NaHCO₃ (0.6 M; pH 8)
- close the PEC filter plate with the bottom-sealing-mat and add 1 mL/well MeCN
- add 1 mL/well of the Reducing Agent, apply top-sealing-mat and shake for 30 min, shake for 90 min when Cys(StBu) has been used
- wash 3x 600 µL/well with water, 3x 600 µL/well with MeCN and discard to remove excess DTT

From this point on, please collect all filtrates in the perforated peptide collection plate, since they hold your product.

- put the peptide collection plate in the vacuum manifold and place the PEC filter plate on the manifold
- add 400 µL/well H₂O/MeCN/TFA (90:8:2)
- let the plate stand in the vacuum manifold for ≥ 4 h (overnight possible)
- collect the peptides by reduced pressure and further elute peptides:
1st: 1.2 mL/well H₂O/MeCN (7:3) + 0.1% TFA
2nd: 1 mL/well H₂O/MeCN (3:7) + 0.1% TFA
(use reduced pressure to decrease elution times)
- take the peptide collection plate out of the vacuum manifold and take your analytical samples
- cover the plate with a paper tissue fixed with rubber bands, freeze in liquid nitrogen **for ≥ 30 min** and lyophilize the peptides

Appendix

Reaction overview



Belyntic's PEC Delta Masses

Table 2: Relevant delta masses during the purification process.

<p>+412.03/414.03 (MH²⁺: +206.52, MH³⁺: +137.67)</p>	<p>PEC-Linker RC+ on peptide</p>
<p>+ 40.05 (MH²⁺: +20.03, MH³⁺: +13.33)</p>	<p>acetone oxime of peptide-linker conjugate, +453.08 Da with PEC-Linker RC+</p>
<p>+ 26.03 (MH²⁺: +13.02, MH³⁺: +8.68)</p>	<p>acetaldehyde oxime of peptide-linker conjugate, +439.06 Da with PEC-Linker RC+</p>
<p>+ 15.99 (MH²⁺: +8.00, MH³⁺: +5.33)</p>	<p>oxidation of methionine residues</p>
<p>+ 12.01 (MH²⁺: +6.01, MH³⁺: +4.00)</p>	<p>formaldehyde oxime of peptide-linker conjugate, +425.04 Da with PEC-Linker RC+</p>
<p>+56.07 (MH²⁺: +28.04, MH³⁺: +18.69)</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 (MH²⁺: +53.03, MH³⁺: +35.35)</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 (MH²⁺: +126.03, MH³⁺: +84.02)</p>	<p>non-cleaved or reattached Pbf; treat purified peptide with Reagent K or another thioanisole-containing TFA cocktail 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 (Cleland's reagent)
DTT	dithiothreitol (Cleland's reagent)
EDT	ethanedithiol
Et ₂ O	diethyl ether
EtOH	ethanol
GdmCl	guanidinium chloride
HFIP	1,1,1,3,3,3-hexafluoro-propan-2-ol
HOBt	1-hydroxybenzotriazole
LC	liquid chromatography
MeCN	acetonitrile
MS	mass spectrometry
MTBE	<i>t</i> -butyl methyl ether
NMP	<i>N</i> -methyl pyrrolidone
Oxyma	ethyl-2-cyano-2-(hydroxyimino) acetate
Pbf	pentamethyldihydrobenzofuran-5-sulfonyl
PEC	Peptide Easy Clean
PhSMe	Thioanisol
RAM	modified Rink-Amide
SDS	safety data sheet
SPPS	solid phase peptide synthesis
<i>t</i> Bu	<i>t</i> -butyl
TFA	trifluoro acetic acid
THF	tetrahydrofuran
TIS	triisopropylsilane
TMSBr	trimethylsilyl bromide



Technical Support

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

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

Safety Data Sheets (SDS)

Safety Data Sheets (SDS) are available on our website <http://belyntic.com>.

Purchaser Notification

Please read the Terms of Sale available on our website <http://belyntic.com>.

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