



Belyntic  
Research Kit

ready to  
use

Research Kit

8x100µmolRC+ K00091  
19.03.2020





Research kit #3-100-0001

# Manual

Catalog number: 100484202

(Reductively Cleavable PEG-Linker Kit)

1023-27-03-0020

### Step 1: PEG-Linker coupling

- 1. Weigh peptide resin in DMF to 35-mg.
- 2. Weigh in the amount of HPL-Buffer and DMSO as shown in Table 1 and combine.

Table 1. PEG-Linker coupling reagent amounts		
PEG-Linker (Kit)	200 mg	0.5 ml
DMSO (Sigma)	400 mg	0.5 ml
HPL-Buffer (100 µl)	100 µl	0.1 ml of HPL-Buffer
HPL-Buffer	100 µl	0.1 ml

- 3. Dissolve the solids in DMF and add DMSO (Table 1).
  - 4. Quickly add the yellow solution to peptide resin.
- Note:** Other common solvents can be used (e.g., Me<sub>2</sub>SO, DMSO or N-methylpyrrolidone (NMP)).
- 5. React for 2-8 hours (overnight is preferred).
  - 6. Wash synthesis resin with 2 x 200 µl DMF.
  - 7. Wash synthesis resin with 2 x 200 µl DMF and dry.

### Step 2: TFA cleavage

- 1. Preheat and prepare proper cleavage cocktail for your peptide (i.e., 50 mM TFA in acetonitrile).
- Note:** To produce a peptide with a C-terminal amide, use a cleavage cocktail containing 100 mM TFA in acetonitrile.
- 2. Add cleavage cocktail to peptide resin and carry out cleavage for 2 hours; only extend cleavage time to save your peptide sequence requires longer cleavage times (e.g., Arg-rich sequences, see PEG-Linker for details).
  - 3. For methionine-containing sequences, store cleavage buffer and add 100 µl TFA to the cleavage cocktail and shake for 10 minutes.
  - 4. Precipitate the peptide in cold methanol and centrifuge at 14,000 x g for 10 minutes.
  - 5. Wash the precipitate once again with cold methanol.

- 6. Dissolve the peptide in 4,500 µl DMF, and make sure that it is fully dissolved.
  - 7. Add 500 µl Buffer 1 (Buffer 1 with 1 M Guanidinium Thiocyanate) to the mixture.
- Note:** Other solvents, DMF, NMP or MeCN may be used as described for DMF, DMSO, or NMP in the cleavage cocktail. For very hydrophobic peptides, acetonitrile may be used for the cleavage cocktail.

### Step 3: Purification

- 1. Filter cartridge pre-filled with 3 ml aqueous heparin suspension and remove supernatant by using a vacuum manifold or by push-pull processing.
  - 2. Wash beads 3x with 1 ml water.
  - 3. Wash beads 3x with 1 ml Buffer 1.
  - 4. Check the cartridge at the bottom and add 1,000 µl DMF to the beads.
  - 5. Dissolve the peptide in 4,500 µl DMF, and make sure that it is fully dissolved.
  - 6. Add 500 µl Buffer 1 (Buffer 1 with 1 M Guanidinium Thiocyanate) to the mixture.
- Note:** Other solvents, DMF, NMP or MeCN may be used as described for DMF, DMSO, or NMP in the cleavage cocktail. For very hydrophobic peptides, acetonitrile may be used for the cleavage cocktail.

### Step 4: Peptide analysis

- 1. Add the dissolved peptide to the HPLC vial.
- 2. Add the dissolved peptide to the HPLC vial.

**Note:** You may pause here and store the resin beads in a sealed vial for later use.

**Note:** Two other modified peptides will show a shift on the HPLC (e.g., 200, 400, 600, 800, 1000) next to the PEG-Linker (e.g., 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000) due to the presence of the PEG-Linker.

### Step 3: Dissolution

- 1. Filter cartridge pre-filled with 3 ml aqueous heparin suspension and remove supernatant by using a vacuum manifold or by push-pull processing.
  - 2. Wash beads 3x with 1 ml water.
  - 3. Wash beads 3x with 1 ml Buffer 1.
  - 4. Check the cartridge at the bottom and add 1,000 µl DMF to the beads.
  - 5. Dissolve the peptide in 4,500 µl DMF, and make sure that it is fully dissolved.
  - 6. Add 500 µl Buffer 1 (Buffer 1 with 1 M Guanidinium Thiocyanate) to the mixture.
- Note:** Other solvents, DMF, NMP or MeCN may be used as described for DMF, DMSO, or NMP in the cleavage cocktail. For very hydrophobic peptides, acetonitrile may be used for the cleavage cocktail.

### Step 4: Peptide analysis

- 1. Add the dissolved peptide to the HPLC vial.
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