



HOOK™ Peptide Coupling Kit (*Amine Reactive*)

A Kit for Coupling Peptides to Carrier Proteins

INTRODUCTION

This kit is designed for the coupling of peptides to carrier proteins, utilizing the primary amine groups of the peptide. This kit utilizes the chemical heterobifunctional crosslinker EDC (1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride). EDC first reacts with the carboxyl groups of the protein/peptide, producing *O*-acylisourea that rapidly reacts with the amine groups of the protein/peptide.

This kit utilizes Tube-O-Reactor™, which allows for the reaction to be performed in a single tube, with no loss of essential reagents and minimum hands on time and effort. This kit is supplied with or without carrier proteins and is suitable for five coupling reactions.

ITEMS INCLUDED

Description	786-067	786-068	786-069	786-070
5X Optimizer Buffer IV	25ml	25ml	25ml	25ml
EDC (1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride), <u>50mg/vial</u>	1 vial	1 vial	1 vial	1 vial
Tube-O-Reactor (Medi) includes 5 Tube-O-Dialyzers™ MEDI (MWCO 8000), 5 Floats, 5 Dialysis Cups and 30 Glass Balls	1	1	1	1
<i>OneQuant</i> ™ Bovine serum albumin (2mg/vial)	N/A	5 vials	N/A	N/A
<i>OneQuant</i> ™ Keyhole Limpet Hemocyanin (2mg/vial)	N/A	N/A	5 vials	N/A
<i>OneQuant</i> ™ HyperCarrier™ (2mg/vial)	N/A	N/A	N/A	5 vials

STORAGE CONDITION

Shipped at ambient temperature. For short-term storage (< 3 months), the complete kit can be stored at 4°C. For long-term storage (>3 months) remove the EDC and store at -20°C protected from moisture. Store other components at 4°C. When stored and used as recommended the kit is stable for one year.

ITEMS NEEDED BUT NOT SUPPLIED WITH KIT

Peptide: Minimum 2mg of peptide of choice to be coupled.

Dialysis Buffer: Suitable storage buffer, e.g. PBS.

PREPARATION BEFORE USE

Prepare 1X Optimizer Buffer™ IV (1ml 5X Optimizer Buffer™ IV in 4ml de-ionized water). The standard protocol requires ~1ml 1X Optimizer Buffer™ IV.

Tube-O-Dialyzers™ are supplied in a preserving solution containing azide. A brief rinse is recommended before use. Place the dialysis cap of the Tube-O-Dialyzer™ on a clean surface or in a clean beaker, the membrane side facing down. Add 200µl 1X Optimizer Buffer™ IV to the cap (membrane) and let it drain away. Keep the Tube-O-Dialyzer™ membrane wet in 1X Optimizer Buffer™ IV until you are ready to use. Do not allow the membrane to dry.



PROTOCOL

NOTE:

- a. To ensure complete coupling, we advise using the recommended quantities of reagents, as these provide a molar excess of peptide to the reactive groups on the carrier proteins.
- b. For peptides insoluble in 1X Optimizer Buffer™ IV, we recommend the use of DMSO (<30%).

1. Resuspend up to 2mg of peptide in 500µl 1X Optimizer Buffer™ IV.
2. Transfer the peptide solution (step 1) to the Tube-O-Dialyzer™ tube.
3. Resuspend 2mg carrier protein of choice in 200µl 1X Optimizer Buffer™ IV and transfer to the Tube-O-Dialyzer containing peptide and mix the content. If using KLH go to step 5.
4. Add 10mg EDC (dry agent) to the Tube-O-Dialyzer™ containing both carrier protein and peptide solution (Step 3). Immediately mix the content.
5. *If using KLH as the carrier protein:*
Weigh 10mg EDC in a 1.5ml centrifuge tube. Add 500µl DMSO and vortex to dissolve the EDC. Add 25µl EDC solution to the solution from step 3 and immediately vortex.
6. Invert the Tube-O-Dialyzer™ and reposition into the Dialysis Cup without any buffer and incubate at room temperature for 2 hours with occasional mixing with inversion.

NOTE: In some cases precipitation may occur. The precipitation occurs due to excessive peptide-carrier protein conjugation. The precipitation may be minimized by reducing the amount of EDC in the reaction. For BSA, HyperCarrier™ and other carrier proteins, the minimum recommended is 0.5mg EDC/mg carrier protein and for KLH 0.05mg EDC/mg carrier protein.

7. Dialyze the reaction for 1-2 hours, with 2-3 changes of dialysis buffer, to remove uncoupled peptide and EDC as following: Add 10ml suitable storage buffer (i.e. PBS) and 5-6 glass balls to the Dialysis Cup. Put a float on the Tube-O-Dialyzer™ and invert into the Dialysis Cup. Put the Dialysis Cup in the Tube-O-Reactor™ and place on a laboratory rotary shaker for the dialysis to proceed. The glass balls in each Dialysis Cup will gently agitate the dialysis buffer and allow rapid and efficient exchange of buffers.

NOTE: a micro stirrer bar and stirrer plate can also be used instead of glass balls and rotary shaker.

8. After dialysis, remove the Tube-O-Dialyzer™ from the float and immediately spin the Tube-O-Dialyzer™ (in up-right position) for 5-6 seconds at 500-1000g. Replace the dialysis cap with a regular cap. The carrier protein-peptide conjugate is ready for use. The carrier protein-peptide conjugate may be stored at -20°C for later use.

RELATED PRODUCTS

1. **HOOK™ Agarose Coupling Kits (Cat.# 786-063 & 786-064):** for the coupling of peptides and proteins to agarose.
2. **HOOK™ Peptide Coupling Kit (Sulphydryl reactive) (Cat.# 786-071, 786-072, 786-073 & 786-074):** for the coupling of peptides to carrier proteins via their sulphydryl residues.
3. **Tube-O-Dialyzer™:** allows dialysis of small samples without having to take the sample out of the tube thus eliminates loss (Medi & Micro size available with 1kDa, 4kDa, 8kDa, 15kDa & 50kDa MW cut off limits).
4. **HOOK™ -Biotin Kit:** supplied with functional biotin agent and a complete set of accessories and buffers for easy labeling.

Note: For other related products, please visit our web site at www.GBiosciences.com.