



213PR-02

G-Biosciences ♦ 1-800-628-7730 ♦ 1-314-991-6034 ♦ technical@GBiosciences.com

A Geno Technology, Inc. (USA) brand name

HOOK™ Peptide Coupling Kit (Sulfhydryl Reactive)

A Kit for Coupling Peptides to Carrier Proteins

(Cat. # 786-071, 786-072, 786-073, 786-074)



think proteins! think G-Biosciences www.GBiosciences.com

INTRODUCTION	3
ITEM(S) SUPPLIED	3
STORAGE CONDITION	3
ADDITIONAL ITEM(S) REQUIRED.....	4
PREPARATION BEFORE USE	4
IMPORTANT NOTES	4
PROTOCOL	5
SUPPLEMENTARY PROTOCOL FOR MODIFICATION OF $-NH_2$ TO $-SH$	5
RELATED PRODUCTS.....	6

INTRODUCTION

This kit is designed for the coupling of peptides to carrier proteins, utilizing a sulfhydryl group in the peptide. This kit utilizes the heterobifunctional crosslinker sulfoSMCC (Sulfosuccinimidyl 4-(*N*-maleimidomethyl) cyclohexane-1-carboxylate). This is a two steps reaction. The *N*-hydroxysuccinimide (NHS) ester in sulfoSMCC reacts first with primary amines on the protein to form covalent amide bonds. In the second step of the reaction the maleimide group reacts with the peptide's sulfhydryl groups to form stable thioether bonds.

If peptides do not contain a sulfhydryl group then primary amines, located at the *N*-terminus and on lysine side chains, may be modified with Traut's reagent to produce sulfhydryl groups (see Supplementary Protocol).

This kit utilizes Tube-O-Reactor™, which allows for the reactions 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.

ITEM(S) SUPPLIED

Description	786-071	786-072	786-073	786-074
5X Optimizer Buffer™ III	25ml	25ml	25ml	25ml
<i>OneQuant</i> ™ sulfoSMCC (5mg/vial)	5 vials	5 vials	5 vials	5 vials
<i>OneQuant</i> ™ Bovine serum albumin (BSA) (2mg/vial)	N/A	5 vials	N/A	N/A
<i>OneQuant</i> ™ Keyhole Limpet Hemocyanin (KLH) (2mg/vial)	N/A	N/A	5 vials	N/A
<i>OneQuant</i> ™ HyperCarrier™ (2mg/vial)	N/A	N/A	N/A	5 vials
Tube-O-Reactor™				
Tube-O-DIALYZER™, Medi (MWCO 8kDa)	5	5	5	5
Floats (Medi)	5	5	5	5
Storage Caps (Medi)	5	5	5	5
Micro Dialysis Cups	5	5	5	5
Glass Balls	50	50	50	50

STORAGE CONDITION

The kit is shipped at ambient temperature. Upon arrival, store the kit 4°C and is stable for 1 year.

ADDITIONAL ITEM(S) REQUIRED

- Peptide: 2mg of peptide of choice to be coupled.
- Dialysis Buffer: Suitable storage buffer, e.g. PBS.

PREPARATION BEFORE USE

- Prepare 1X Optimizer Buffer™ III (1ml 5X Optimizer Buffer™ III in 4ml de-ionized water). The standard protocol requires ~1ml 1X Optimizer Buffer™
- Tube-O-Dialyzer™ 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™ to the cap (membrane) and let it drain away. Keep the Tube-O-Dialyzer™ membrane wet in 1X Optimizer Buffer™ until you are ready to use. Do not allow the membrane to dry.

IMPORTANT NOTES

- To ensure complete coupling, we advise using the recommended quantities of reagents, as these provide a molar excess of peptide to the maleimide groups on the carrier proteins.
- For peptides insoluble in 1X Optimizer Buffer III, we recommend the use of DMSO (<30%).
- The kit is designed for the coupling of protein and peptides to agarose through free sulfhydryl residues. Ellman's reagent (Cat#BC87) can determine the presence of free sulfhydryl groups. If oxidized, the proteins and peptides can be reduced with β-mercaptoethanol (Cat.# BC98), DTT (Cat.#786-227) or TCEP (Cat.# 786-230). Following reduction, the reducing agent must be removed and we recommend using a gel filtration spin column ((SpinOUT™, Cat # 786-170, 786-171). This method is not suitable for small peptides (MW < 1500) and we recommend Reducing Resin (Immobilized TCEP) (Cat. 786-822) for the reduction of peptides.

PROTOCOL

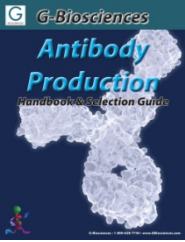
1. For BSA and HyperCarrier™, resuspend 2mg carrier protein in 200µl 1X Optimizer Buffer™ III by gentle pipetting. For KLH, briefly centrifuge to collect the KLH solution to the bottom of the vial and proceed to the next step.
2. Resuspend a vial of *OneQuant*™ sulfoSMCC in 50µl DMSO. Vortex and gentle pipette to create a homogenous suspension (Note: sulfoSMCC will not dissolve). Add 10µl sulfoSMCC suspension to the carrier protein solution from step 1 and immediately vortex. Incubate at 37°C for 30 minutes.
3. Add carrier protein:sulfoSMCC to a Tube-O-Dialyzer™ tube. Invert the Tube-O-Dialyzer™ tube and position in the Mini-Dialyzer containing 10ml PBS. Dialyze the reaction for 1-2 hours, with 2-3 changes of dialysis buffer.
4. Immediately before use, dissolve 2-10mg sulfhydryl containing peptide in 500µl 1X Optimizer Buffer™ III.
5. After dialysis in step 3, 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. Gently loosen the cap and introduce the peptide solution (Step 4) to the Tube-O-Dialyzer™ containing protein-sulfoSMCC conjugate. Mix the content.
6. Invert the Tube-O-Dialyzer™ tube and position in the Mini-Dialyzer tank without any buffer. Incubate at room temperature for 30 minutes. Mix the reaction and incubate for another 1 hour at 4°C.
7. Add 10ml suitable storage buffer, i.e. PBS, to the Mini-Dialyzer tank and dialyze the reaction for 1-2 hours, with 2-3 changes of dialysis buffer, to remove uncoupled peptides.
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.

SUPPLEMENTARY PROTOCOL FOR MODIFICATION OF -NH₂ TO -SH

1. Suspend the peptide or proteins to be thiolated in 50mM triethanolamine, 0.15M NaCl, 1mM EDTA, pH8.0.
2. Add a 3-4 fold molar excess of Traut's reagent (Cat# BC95).
3. Incubate the reaction for 45 minutes at room temperature, under nitrogen gas.
4. Separate the thiolated products from unreacted Traut's reagent, using a SpinOUT™ column (SpinOUT™ GT-600 (Cat# 786-170)) or by dialysis.

RELATED PRODUCTS

Download our Antibody Production Handbook.



<http://info.gbiosciences.com/complete-Antibody-Production-handbook>

For other related products, visit our website at www.GBiosciences.com or contact us.

Last saved: 1/22/2014 CMH

This page is intentionally left blank



www.GBiosciences.com