



Title: *Preparing Antisera to Peptides*

No: RTLTP-GL-Ab-4

Location:
Old CCRC Tripp Lab

Approval Date:
10 September 2004

Supersedes Date:

Materials:

Page 1 of 2

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| <ul style="list-style-type: none"> •Lab coat •Gloves •Centrifuge •Incubator 37°C •Keyhole limpet hemocyanin (KLH) •0.01M Potassium phosphate buffer, pH 7.0 •Beakers (dialysis) •0.1M HCL •0.1M NaOH | <ul style="list-style-type: none"> •Cys-containing synthetic peptides: HMPV-F (1-6)and HMPV-G (1) •0.05M Potassium phosphate buffer, pH 6.0 •Phosphate-buffered Saline (PBS) •m-maleimidobenzoyl-N-hydroxysuccinimide ester in dimethylformamide (MBS/DMF)* <p>*Prepared 1 hour prior to use</p> | <ul style="list-style-type: none"> •Dialysis tubing (10,000 MWCO), 10' 75mm (3mL) •Dialysis clamps •15mL glass test tube •Ellmans Reagent | <ul style="list-style-type: none"> •PD-10 column, pre-packed •Lyophilizer •Pipettes •Pipetteman •Pipette Aid •Pipetteman tips •pH meter •-20°C cold storage •4°C cold storage |
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NOTE: Tris buffers should not be used in this procedure.

Procedure:

1. Dissolve 5 mg KLH in 0.5 ml of 0.01 M phosphate buffer, pH 7.0. Place in dialysis tubing and dialyze against 4 liters of 0.01 M phosphate buffer, pH 7.0, overnight at 4°C. Transfer dialyzed solution into a 10 ´ 75-mm glass test tube.
2. Add 70 ul MBS/DMF (prepared 1 hour prior to use) to dialyzed KLH solution. Stir gently with magnetic stirbar 30 min at room temperature.

NOTE: Pierce sells maleimide-activated carrier proteins (KLH, BSA, and ovalbumin) that can be used to couple Cys-containing peptides, as well as immunogen conjugation kits for these activated proteins.

3. Pre-equilibrate a PD-10 column by washing with 50 ml of 0.05 M phosphate buffer, pH 6.0, and load the KLH reaction mixture (from step 2). Elute the column with 0.05 M phosphate buffer, pH 6.0, and collect about twenty 0.5-ml fractions. Read the A₂₈₀ of the fractions.

Preparing Antisera to Peptides
Page 2 of 2

The first peak represents MB/KLH conjugate and the second peak represents free MBS. The MB/KLH peak is readily identified by its cloudiness.

4. Pool the MB/KLH conjugate fractions and place them in a 15-ml test tube (does this need to be glass?).
5. Dissolve 5 mg synthetic peptide in 1 ml PBS.

If the peptide is not soluble in PBS, it can be dissolved in 6 M guanidine×HCl/0.01 M phosphate buffer, pH 7.0.

6. Add the peptide solution to the MB/KLH conjugate (from step 4). Check pH and adjust to 7.3 with 0.1 M HCl or 0.1 M NaOH. Stir 3 hr at room temperature with magnetic stirbar.
7. Dialyze against 4 liters of water overnight at 4°C. Replace with fresh water and dialyze for 34 hr at 4°C. Save an aliquot for step 8, maintaining conjugate at 4°C. Lyophilize the remainder and store at -20°C. *This should provide enough immunogen*
8. Ellman's reagent can be used to determine coupling efficiencies.
9. Immunize mice to obtain antiserum.

Author	Management Approval/Date	Effective Date