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# Peptide fragment (579-601 from HIV-gp41) conjugated to keyhole limpet haemocyanin to be used as HIV immunogen.

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**We use this protocol and it's working**

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## Abstract

Chemical synthesis facilitates the generation of peptides which are exceedingly difficult to express in bacteria, peptide/protein backbone modification, the incorporation of unnatural amino acids, and the production or synthesis of D-proteins.
The C-terminal cysteine can be added to the amino acidic sequences of HIV peptides (fragment 579-601 of the HIV-gp41 [1]. These peptide fragments were dimerized by cysteine oxidation with dimethyl-sulfoxide [2] to facilitate their conjugation to keyhole limpet hemocyanin that acts as a carrier protein.

Reference:

1. McPhee DA, Kemp BE, Cumming S, Stapleton D, Gust ID, Doherty RR. Recognition of envelope and tat protein synthetic peptide analogs by HIV positive sera or plasma. *FEBS Lett*. 1988;233(2):393-396. doi:10.1016/0014-5793(88)80468-x.
2. Tam JP, Wu CR, Liu w, Zhang JW (1991) Disulfide bond formation in peptides by dimethyl sulfoxide. Scope and applications. J Am Chem Soc 113: 66576662.

## Guidelines

The Protocol has a high level of reproducibility and has worked for many other HIV peptides.

## Materials

MATERIALS

- 10mg KLH (Keyhole Limpet Hemocyanin) (Immunological Grade) **G-Biosciences Catalog #**786-088
- Glutaraldehyde, 50% solution **Bio Basic Inc. Catalog #**G0875.SIZE.100ml
- Peptide 579-601 of HIV-gp41
- Fragment 579-601 of gp41

## Troubleshooting

## Safety warnings

Glutaraldehyde presents serious side effects including skin irritation, nausea, headache, and shortness of breath.

1 These peptide fragment (579-601 from HIV-gp41) is dimerized by cysteine oxidation with dimethyl-sulfoxide. The HIV peptide is dissolved in 5% acetic acid to a final concentration of 5.1 mg/ml.

2 The pH of the medium is adjusted to 6 with 1 M (NH4)2CO3.

3 Dimethyl-sulfoxide is added to 20% of the final volume, and after four hours at room temperature (RT), the solute is extracted.

4 Then, the peptide is dissolved in 3 ml 5% trifluoroacetic acid and precipitated with 35 ml cold ether.

5 The precipitate is dialyzed against 1.2 liters of deionized water, pH 7 at 4°C overnight.

6 The fragment 579-601 of gp 120 was conjugated by the glutaraldehyde method.

7 One mg of keyhole limpet hemocyanin (KLH) is dissolved in 2 ml 0.1 M borate buffer (1.24 g boric acid, 1.90 g sodium tetraborate, pH 10, in 500 mL deionized water).

8 In a 20 ml glass tube by gentle stirring 1 µmol of the HIV synthetic peptide and 0.2 mL 0.3% glutaraldehyde solution (ACS reagent grade, pH 5.5, Sigma-Aldrich) are slowly mixed at RT and left to stand for 2 hrs.

9 When a yellow coloration is observed this indicates that the conjugation process is successful.

10 To blocking the excess of glutaraldehyde, 0.26 ml of 1 M glycine (Sigma-Aldrich) is added.

11 The mix is left for 32 min at RT.

12 The HIV-hemocynin conjugate is then dialyzed against 1.3 liters 0.1 M of borate buffer, pH 8.4 through the night at 4°C.

13 Then the 0.1 M borate buffer is used to dialyze the preparations for 8 hrs at 4°C.