



Aug 10, 2020

# 🌐 Conjugation of Keyhole limpet haemocynin to Peptide 254-274 of HIV gp-120 as immunogen.

DOI

[dx.doi.org/10.17504/protocols.io.bjh7kj9n](https://dx.doi.org/10.17504/protocols.io.bjh7kj9n)

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**Protocol Citation:** Angel A Justiz-Vaillant 2020. Conjugation of Keyhole limpet haemocynin to Peptide 254-274 of HIV gp-120 as immunogen.. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.bjh7kj9n>

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**Protocol status:** Working

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

**Created:** August 10, 2020

**Last Modified:** August 10, 2020

**Protocol Integer ID:** 40223

## Abstract

Chemical synthesis facilitates the generation of peptides which are difficult to express in bacteria. The fragment 254-274 of the human immunodeficiency virus (HIV) is critical for infectivity of the virus and induction of antibody neutralization (Ho et al, 1988).

Boudet et al, 1995 demonstrated that a synthetic peptide derived from the third variable domain of the HIV-1 gp-120 when used as immunogen was able to induce an antibody response to multiple (up to six) HIV strains.

## Reference

Ho DD, Kaplan JC, Rackauskas IE, Gurney ME. Second conserved domain of gp120 is important for HIV infectivity and antibody neutralization. *Science*. 1988;239(4843):1021-1023. doi:10.1126/science.2830667.

Boudet F, Keller H, Kieny MP, Thèze J. Single peptide and anti-idiotypic based immunizations can broaden the antibody response against the variable V3 domain of HIV-1 in mice. *Mol Immunol*. 1995;32(7):449-457. doi:10.1016/0161-5890(95)00007-2

## Guidelines

The Protocol has a high level of reproducibility and has worked for many other HIV peptides. It has been used in peer-review research.

## 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 421-438 HIV-gp120

- 1 The fragment 254-274 of gp 120 was conjugated by the glutaraldehyde method.
- 2 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).
- 3 In a 20 ml glass tube by gentle stirring 1  $\mu$ mol 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.
- 4 When a yellow coloration is observed this indicates that the conjugation process is successful.
- 5 To blocking the excess of glutaraldehyde, 0.26 ml of 1 M glycine (Sigma-Aldrich) is added.
- 6 The mixture is left for 31 min at RT.
- 7 The HIV-hemocynin conjugate is then dialyzed against 1.1 liter 0.1 M of borate buffer, pH 8.3 through the night at 4°C.
- 8 Then use borate buffer again to dialyze the preparation for 8 hrs at 4°C.
- 9 The dialysates is stored at 4°C.