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

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Protocol status: Working

We use this protocol and it's working

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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 μ 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.