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Detection of anti- keyhole limpet hemocynin (anti-KLH) antibodies by double immunodiffusion (Ouchterlony) technique.

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We use this protocol and it's working

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Abstract

Keyhole limpet hemocyanin (KLH) is a cooper-containing protein comprising of subunits with MW of 400 kDa. This protein is found in the hemolymph of the sea mollusk Megathura crenulata. It has the ability to enhance the host's immune response by interacting with monocytes, T cells and macrophages. KLH has been used primarily as a carrier for vaccines and antigens [1]. It was found that chicken immunized with KLH bound peptide raised an anti-KLH immunoresponse [2]. This can be tested by a single method such as the Ouchterlony technique.

Reference

1. Aarntzen EH, de Vries IJ, Göertz JH, et al. Humoral anti-KLH responses in cancer patients treated with dendritic cell-based immunotherapy are dictated by different vaccination parameters.*Cancer Immunol Immunother*. 2012;61(11):2003-2011. doi:10.1007/s00262-012-1263-z

2. Justiz Vaillant AA, Anderson MF, Smikle M, Wisdom B, Mohammed W, et al. (2013) Development of Anti HIV Gp120 and HIV Gp41 Peptide Vaccines. J Vaccines Vaccin 4: 206. doi: 10.4172/2157-7560.1000206

Materials

MATERIALS

10mg KLH (Keyhole Limpet Hemocyanin) (Immunological Grade) **G-Biosciences Catalog #**786-088

Troubleshooting

1 Detection of anti-keyhole limpet hemocynin antibodies by double immunodiffusion is carried out.

2 Briefly, 1% agarose gels are prepared and wells cut into the gel using a template.

3 Initially, aliquots of 25 µl each of KLH in concentration of 1 mg/ml are applied to the centre well.

4 The peripheral wells are filled with 25 µl of isolated chicken IgY (1 mg/ml) in PBS pH 7.4.

5 The gels are incubated at RT for 48–72 hours.

6 After that the gels are examined for precipitin lines.

7 An anti-KLH developed in cat is included as positive control and turtle serum as a negative control.

8 The positive results are taken as the presence of precipitin line/s and negative results, the absence of precipitin lines.