Aug 15, 2020

Detection of anti- SpA antibodies in egg white tested by double immunodiffusion (Ouchterlony) technique.

DOI
dx.doi.org/10.17504/protocols.io.bjsrknd6

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Protocol Citation: Angel A Justiz-Vaillant 2020. Detection of anti- SpA antibodies in egg white tested by double immunodiffusion (Ouchterlony) technique.. **protocols.io https://dx.doi.org/10.17504/protocols.io.bjsrknd6**

Protocol status: Working
We use this protocol and it's working

Created: August 15, 2020

Last Modified: August 15, 2020

Protocol Integer ID: 40497

Keywords: cancer immunol immunother, based immunotherapy, antigen, double immunodiffusion, vaccine, immune response, carrier for vaccine, hemolymph of the sea mollusk megathura crenulata, bound peptide, dendritic cell, different vaccination parameter, containing protein comprising, protein, hiv gp41 peptide vaccine, protein comprising of subunit, sea mollusk megathura crenulata, macrophage

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-SpA antibody in the egg white of eggs from chicken vaccinated with SpA 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 protein-A (SpA) in concentration of 1 mg/ml are applied to the centre well.

4 The peripheral wells are filled with 25 µl of 1:4 dilutions of egg whites from eggs of chicken immunized with SpA.

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

6 After that the gels are examined for precipitin lines.

7 An anti-SpA antibody developed in rat 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.