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Imm-Link™ Ovalbumin Immunogen Kit (for amine groups)

Applicable to:

451-0001 1 x 2mg Ovalbumin Immunogen kit (for amines)

451-0500 3 x 2mg Ovalbumin Immunogen kit (for amines)

Release 3 06/01/2016

Introduction

The Imm-Link™ amine conjugation kit allows amine-containing haptens to be conjugated to a carrier protein simply by adding a solution of the hapten to a proprietary lyophilised mixture containing both the carrier protein and all of the chemicals required to form the conjugate. Upon dissolution of the Imm-Link™ mixture proprietary chemicals in the mixture become activated, resulting in the coupling of the hapten to the carrier protein in a gentle and controlled process. The hands-on time to set up the conjugation reaction is typically 20-30 seconds.

Once the conjugation reaction is complete the hapten-carrier conjugate is dialysed using the supplied dialysis cartridge to remove unwanted by-products with ease from the conjugation reaction. The hapten conjugate can then be used for the purpose of antibody production. The design of the dialysis cartridge ensures that the hapten conjugate is recovered in high yield.

Kit contents

1 or 3 glass vial(s) of Imm-Link™ mix

1 or 3 vial(s) of Imm-Link™ Amine Modifier reagent

1 or 3 dialysis cartridge(s)

1 or 3 250ml bottle(s) of 10X dialysis buffer

Shipping and storage

The kit is shipped at ambient temperature. Upon receipt store the plastic pot and its components at -20°C. The other components should be stored at room temperature.

Considerations before use

Sample buffer

Ideally, the hapten should be salt-free or in a 10-50mM buffer in the pH range 6.5 to 8.5.

See Appendix for further guidance on buffers and compatible additives.

Amount and volume of hapten

Each molecule of ovalbumin contains between 5 and 10 potential sites for attachment. The absolute amount of material that can be bound will depend on the size of the individual hapten.

Small molecules, peptides or small proteins may be conjugated as long as the correct functional groups are present.

The volume of the solution of the molecule to be conjugated to the carrier protein should be between 0.4 and 1ml.

Instructions

1. Setting up conjugation reactions:

1.1 Before you add the hapten to the Imm-Link™ mix, add 1µl of Imm-Link™ Amine Modifier reagent to each 10µl of hapten to be labeled. Mix gently.

1.2 Remove the cap from the vial of Imm-Link™ mix and pipette the sample (with added modifier) directly onto the lyophilised material. A maximum conjugation volume of 1ml is recommended.

1.3 Resuspend gently by withdrawing and redispensing the liquid once or twice using a pipette.

1.4 Place the cap back on the vial and leave the vial standing for 4 hours at room temperature (20-25°C). Alternatively, and sometimes more conveniently, conjugations can be set up and left overnight at room temperature. The longer incubation time does not have any negative impact on the conjugate.

2. Dialysis of the conjugate

2.1 Prepare the dialysis buffer by diluting the 10X stock to 1X with deionised/distilled water.

2.2 Remove the plastic outer packaging from the dialysis cartridge.

2.3 Remove the black screw cap lid from the top of the dialysis cartridge.

2.4 Pipette the conjugate into the dialysis cartridge and replace the black lid.

2.5 Place the flotation ring on the top of the cartridge and place the cartridge in the dialysis buffer.

2.6 The conjugate should be dialysed against 1 litre of 1x dialysis buffer for at least 4 hours, and then against a fresh 1 litre of dialysis buffer, for at least 4 hours.

Note: A longer dialysis will not affect the conjugate quality; a combination of dialysis overnight and during the day i.e. ~16 hours and then ~7 hours is convenient and perfectly acceptable.

2.7 After dialysis, unscrew the black cap and recover the dialysed conjugate using a pipette.

2.8 The conjugate is now ready to use.

Storage of conjugates

If you are not using the conjugate immediately, store frozen at -20°C or -70°C in aliquots until required. Avoid multiple freeze thaw cycles if possible.

Appendix

Compatibility of buffers and buffer additives

Note: The advice below relates specifically to the subset of Imm-Link™ products that are used for conjugation of amine-containing haptens. The requirements for other Imm-Link™ products may be different.

Since a link is formed between an amine group on the hapten and the carrier, there must be no other primary amines in the solution of the hapten, as these amines may compete with the intended hapten-carrier conjugation reaction.

If the hapten is dissolved in a buffer, MES, MOPS, HEPES, and phosphate are suitable buffers for Imm-Link™ kits for primary amines. Common non-buffering salts (e.g. sodium chloride), chelating agents (e.g. EDTA), and sugars may be present, as they have no effect on conjugation efficiency. Azide (0.02-0.1%) has little or no effect.

You should avoid buffer additives that are nucleophilic, as these may react with Imm-Link™ chemicals and cause interference. You should therefore avoid thiols (e.g. mercaptoethanol and DTT) and primary amines (except of course those present on your hapten).

For further information about Innova's products please see our website: https://www.innovabiosciences.com

Technical Support Team
Tel: +44(0)1223 496170
E-mail: technical.enquiries@innovabiosciences.com