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462-0001 Protocol

Imm-Link BSA (Sulfhydryl) Immunogen labeling Kit Conjugation Protocol (462-0001)

1. Introduction
The imm-Link(TM) Sulfhydryl conjugation kit allows sulfhydryl-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(TM) 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.

2. Instructions
2.1 Storage and components
The kit is shipped at ambient temperature. Upon receipt store the lyophilized component (glass vial) at -20 degrees C. The other components should be stored at room temperature.

Kit contents:
Glass vial(s) of imm-Link(TM) mix (1 or 3 vials, depending on pack size)
1 vial of imm-Link(TM) Sulfhydryl Modifier reagent Dialysis cartridge(s) (1 or 3 depending on pack size)
250ml of 10X dialysis buffer (1 or 3 bottles depending on pack size)

2.2 Considerations before use
2.2.1 Sample buffer
Ideally, the hapten should be salt-free or in a 10-50mM buffer. The buffer should be free of primary amines and in the pH range 6.5 to 8.5. Appendix 1 gives further guidance on buffers and compatible additives.
2.2.2 Amount and volume of hapten
Each molecule of BSA contains between 15 and 20 potential sites of 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 assuming that 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.

2.3 Setting up conjugation reactions
2.3.1. Before you add the hapten to the imm-Link(TM) mix, add 1ul of imm-Link(TM) Sulfhydryl Modifier reagent to each 10ul of hapten to be labeled. Mix gently.
2.3.2. Remove the cap from the vial of imm-Link(TM) mix and pipette the sample (with added modifier) directly onto the lyophilised material. Resuspend gently by withdrawing and redispensing the liquid once or twice using a pipette.
2.3.3. Place the cap back on the vial and leave the vial standing for 4 hours at room temperature (20-25 degrees C). Alternatively, and sometimes more conveniently, conjugations can be set up and left overnight. The longer incubation time does not have any negative impact on the conjugate.

2.4 Dialysis of the conjugate
2.4.1 Prepare the dialysis buffer by diluting the 10X stock with deionised/distilled water.
2.4.2 Remove the plastic outer packaging from the dialysis cartridge.
2.4.3 Remove the black screw cap lid from the top of the dialysis cartridge.
2.4.4. Pipette the conjugate into the dialysis cartridge and replace the black lid.
2.4.5 Place the flotation ring on the top of the cartridge and place in the cartridge in the dialysis buffer.
2.4.6 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: you cannot dialyse for too long; a combination of dialysis overnight and during the day i.e. ~16 hours and then ~7 hours is convenient and perfectly acceptable.
2.4.7 After dialysis, unscrew the black cap and recover the dialysed conjugate using a pipette.
2.4.8 The conjugate is now ready to use

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

Appendix 1. Compatibility of buffers and buffer additives
Note: The advice below relates specifically to the subset of imm-Link(TM) products that are used for conjugation of Sulfhydryl-containing haptens. The requirements for other imm-Link(TM) products may be different.

If the hapten is dissolved in a buffer, MES, MOPS, HEPES and phosphate are suitable buffers for imm-Link(TM) kits for sulfhydryl groups. 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(TM) chemicals and cause interference. You should therefore avoid thiols (e.g. mercaptoethanol and DTT).