Innova Biosciences
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EUROPIUM Conjugation Kit – Mini Vial Conjugation

Applicable to:
1200-0040, 1200-0100

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Introduction

The EUROPIUM (Eu) Conjugation Kit allows antibodies or proteins to be quickly and easily conjugated to high quality 200 nm Europium chelate microspheres. The particles have been specially treated to enhance the handling of the beads and permit easy covalent attachment of antibodies and other proteins.

The Eu microspheres in this kit are freeze dried. The conjugation reaction is initiated simply by reconstituting the dry mixture with your antibody, which becomes attached (via lysine residues) to the specially treated surface.

It takes 30 seconds to set up the conjugation, the hands-on time for the conjugation procedure is about 3 minutes and the conjugate is ready to use within 35 minutes. You simply pipette the biomolecule into a vial containing the particles, allow the conjugation to occur and then centrifuge to buffer exchange (Figure 1).

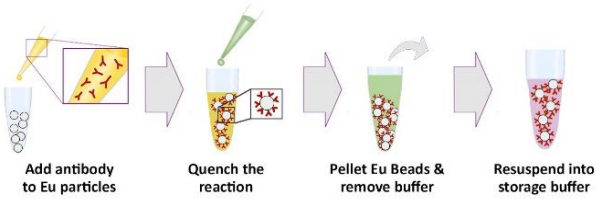

Figure 1. EUROPIUM Conjugation Process

The surface treatment makes the particles resistant to aggregation and the Eu fluorescence signal allows you to reach a higher sensitivity in your immunoassay.
Additionally, unlike passive methods the conjugation procedure has only a weak dependence on the isoelectric point of the antibody. Consequently, extensive trials at different pH values are not required; all antibodies can be conjugated at one of two pHs, both of which are supplied in this kit (Reaction Buffers A and B).
We recommend that your antibody is in 10 – 50 mM MES, HEPES or MOPS at pH 6 – 7 (with no other components e.g. salt or azide) prior to conjugation. See the "Buffer Considerations" section of this protocol for more details and advice if your antibody is in another buffer.

Kit contents

4 or 10 Mini vials of 200 nm Europium (dependent on kit)
Each Mini vial gives sufficient conjugate for up to 400 Lateral Flow tests and 1 x 96 wells microplate assays.

1 vial of 1x Reaction Buffer A
1 vial of 1x Reaction Buffer B
1 vial of 10x Europium Quencher
1 vial of 1x Resuspension Buffer

Shipping conditions

The kit is shipped at ambient temperature in a tamper-evident polypropylene container.

Store the kit at -20°C upon receipt.

All the buffers and the Quencher can be stored at either 4°C or -20°C.

Instructions

1. Allow all of the reagents to warm to room temperature.
2. Dilute your stock antibody to 0.1 mg/ml with Reaction Buffer A or B. 45 µl will be sufficient to conjugate one Mini vial. *Note: If this is the first test of an antibody we advise carrying out the reaction twice, once with Reaction Buffer A and once with Reaction Buffer B to find the optimal pH. If you also wish to examine the effect of varying the amount of antibody, make different antibody dilutions.*
3. Add 40 µl of the 0.1 mg/ml antibody to the Mini vial, and reconstitute the Eu particles by gently and thoroughly pipetting up and down. Incubate the reaction for exactly 15 minutes at room temperature.
4. Dilute sufficient 10x Eu Quencher with deionised water. For one Mini vial you need exactly 1 ml so we advise you to make 1.2 ml 1x Eu Quencher per vial i.e. 120 µl 10x Eu Quencher + 1080 µl water.
5. After 15 minutes, add 1 ml of 1X Eu Quencher to stop the reaction, and mix by inverting several times.
6. Leave the reaction to quench for 5 minutes at room temperature, then transfer into a microcentrifuge tube and spin at 13,800 g for 8 minutes. Remove ~850 µl of the supernatant and without resuspending the pellet spin for 2 more minute at 13,800 g. Remove the rest of the supernatant.

7. Gently tap the pellet first and then add 40 µl of Resuspension Buffer. Thoroughly resuspend the pellet by pipetting up and down for at least 90 seconds.
8. You now have 40 µl 1 % conjugate.
9. Dilute the conjugate as required for your application.

We recommend to dilute the conjugate to 0.0025%-0.005%, although you may need to optimize this according to your application.

Amount and volume of antibody

The optimum amount of antibody (which will influence the number of antibody molecules per particle) depends on the size of the particles (surface area) and on the application; you may need to conjugate different amounts of antibody to optimize your assay. We recommend testing with 0.1 mg/ml in the first instance, although slightly lower or higher concentrations can be explored to optimize performance in your particular application. Before testing antibody concentrations above 0.1 mg/ml we advise you to use our Antibody Concentration & Clean Up Kit for LATEX and EUROPIUM to remove interfering buffer components. This is due to the increase in contamination that will occur when conjugating using a larger volume of the stock antibody. Do not alter the reaction volume of 40 µl as this will reduce conjugation efficiency.

Storage of conjugates

Initial conjugate storage at 4°C is recommended. The Resuspension Buffer added at the end of the conjugation reaction is a good storage buffer. Do not store the conjugate at -20°C. The determining factor for conjugate stability will be the antibody itself, as it will be first to degrade. Therefore as long as your antibody is stable, the conjugate will be stable as well.

Buffer considerations

Buffer composition is crucial for the efficiency of the conjugation and so for the sensitivity of the conjugated Eu particles. Our advice is to carry out the conjugation only from stock antibodies that are at least 1 mg/ml in 10 mM-50 mM MES, HEPES or MOPS at pH 6-7 (with no other components as Azide or salt). You can easily achieve the desired antibody buffer conditions using our Antibody Concentration and Clean Up kit, developed for LATEX and EUROPIUM conjugation kits. This kit will quickly and simply purify your antibody into Reaction Buffer A and/or B. Please see the kit protocol for more details.

Please see the table below for more details of compatible and incompatible buffer components for the Eu conjugation kit, and those that can be removed by the Antibody Concentration & Clean Up Kit for LATEX and EUROPIUM. Proteins cannot be removed by the Antibody Concentration & Clean Up Kit for LATEX and EUROPIUM, instead you can use our AbPure™ kits. If the antibody to be conjugated has been purified using a compatible AbPure™ kit, please see the kit protocol for minimum antibody concentrations prior to conjugation.

Buffer components	EUROPIUM Conjugation Kit can tolerate	Concentration & Clean Up Kit can remove
pH 6 - 7	✓	✓
pH < 6 and > 7	✗	✓
Amine free buffer (≤50 mM) *(e.g. MES, MOPS, HEPES)*	✓	✓
Amine free buffer (≥50 mM) *(e.g. MES, MOPS, HEPES)*	✗	✓
Salt	✗	✓
Sodium Azide	✗	✓
Sugars	✓	✓
Glycerol	✓	✓
Thiomersal	✗	✓
Thimerosal	✗	✓
Merthiolate	✗	✓
BSA	✗	✗[1]
Gelatin	✗	✗[1]
Tris	✗	✓
Glycine	✗	✓
Carboxylic acids *(e.g. EDTA, Citrate)*	✗	✓
Nucleophilic components *(Primary amines e.g. amino acids or ethanolamine and thiols e.g. mercaptoethanol or DTT)*	✗	✓

[1] We recommend using our AbPure™ kits if the antibody to be conjugated contains other unwanted proteins such as BSA or Gelatin.

FAQs

For technical enquiries or for further information please get in touch at www.innovabiosciences.com/contact-us.html.