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ab269889 Europium Conjugation Kit

A product of Expedeon, an Abcam company

Applicable to Expedeon product codes 1200-0040, 1200-0100.

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Europium Conjugation Kit datasheet:
www.abcam.com/ab269889
(use www.abcam.cn/ab269889 for China, or www.abcam.co.jp/ab269889 for Japan)

For the Conjugation of Antibodies or Proteins to Europium.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Europium Conjugation Kit (ab269889) allows antibodies or proteins to be quickly and easily conjugated to high quality 200 nm Europium (Eu) 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 the 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.

The surface treatment makes the particles resistant to aggregation and the Eu fluorescence signal allows a higher sensitivity to be reached in immunoassays such as lateral flow assays.

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).

2. Materials Supplied and Storage

Store kit at -20°C immediately on receipt. All the buffers and the Quencher can be stored at either +4°C or -20°C. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Avoid repeated freeze-thaws of reagents.

Item	Quantity			Storage temperature
	4 x 4 µg	10 x 4 µg	1 x 40 µg	
200 nm Europium vial	4 vials	10 vials	1 vial	-20°C
1x Reaction Buffer A	1 vial	1 vial	1 vial	+4°C or -20°C
1x Reaction Buffer B	1 vial	1 vial	1 vial	+4°C or -20°C
10x Europium Quencher	1 vial	1 vial	1 vial	+4°C or -20°C
1x Resuspension Buffer	1 vial	1 vial	1 vial	+4°C or -20°C

Reagents are ready to use as supplied.

3. Technical Considerations

3.1 Amount of volume of antibody to be conjugated:

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 (ab269889) 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 (if using the 4 x 4 µg or 10 x 4 µg kits) or 400 µL (if using the 1 x 40 µg kit) as this will reduce conjugation efficiency.

3.2 Buffer considerations before conjugation:

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 (ab269965). 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 Europium conjugation kit (ab269889), and those that can be removed by the Antibody Concentration & Clean Up Kit for Latex and Europium (ab269965).

Buffer components	Europium Conjugation Kit can tolerate
pH 6 - 7	Yes
pH < 6 and > 7	No
Amine free buffer (≤50 mM) *(e.g. MES, MOPS, HEPES)*	Yes
Amine free buffer (≥50 mM) *(e.g. MES, MOPS, HEPES)*	No
Salt	No
Sodium Azide	No
Sugars	Yes
Glycerol	Yes
Thiomersal	No
Thimerosal	No
Merthiolate	No
BSA	No
Gelatin	No
Tris	No
Glycine	No
Carboxylic acids *(e.g. EDTA, Citrate)*	No
Nucleophilic components *(Primary amines e.g. amino acids or ethanolamine and thiols e.g. mercaptoethanol or DTT)*	No

4. Assay Procedure

Please follow "Procedure A" below for the 4 x 4 µg or the 10 x 4 µg kits, or "Procedure B" for the 1 x 40 µg kit.

Procedure A:

4.1 Allow all of the reagents to warm to room temperature.

4.2 Dilute your stock antibody to 0.1 mg/mL with Reaction Buffer A or B. 45 µL will be sufficient to conjugate one 200 nm Europium vial.

*Δ **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.*

4.3 Add 40 µL of the 0.1 mg/mL antibody to the 200 nm Europium vial and reconstitute the Eu particles by gently and thoroughly pipetting up and down. Incubate the reaction for exactly 15 minutes at room temperature.

4.4 Dilute sufficient 10x Europium Quencher with deionised water. For one 200 nm Europium vial you need exactly 1 mL so we advise you to make 1.2 mL 1x Europium Quencher per vial i.e. 120 µL 10x Europium Quencher + 1080 µL water.

4.5 After 15 minutes, add 1 mL of 1X Europium Quencher to stop the reaction, and mix by inverting several times.

4.6 Leave the reaction to quench for 5 minutes at room temperature, then transfer into a microcentrifuge tube and spin at 13,800 x *g* for 8 minutes. Remove ~850 µL of the supernatant and without resuspending the pellet spin for 2 more minutes at 13,800 x *g*. Remove the rest of the supernatant.

4.7 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.

4.8 You now have 40 µL 1 % conjugate.

4.9 Dilute the conjugate as required for your application.
*Δ **Note**: We recommend to dilute the conjugate to 0.0025%-0.005%, although you may need to optimize this according to your application.*

Procedure B:

4.1 Allow all of the reagents to warm to room temperature.

4.2 Dilute your stock antibody to 0.1 mg/mL with Reaction Buffer A or B. 450 µL will be sufficient to conjugate one Europium vial.

*Δ **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. You may also wish to examine the effect of varying the amount of antibody, by testing different antibody dilutions. The 10 reaction kit (ab269989) is designed for such optimization experiments.*

4.3 Add 400 µL of the 0.1 mg/mL antibody to the Europium vial and reconstitute the Eu particles by gently and thoroughly pipetting up and down. Incubate the reaction for exactly 15 minutes at room temperature.

4.4 Make 12 mL 1x Europium Quencher: 1.2 mL 10x Europium Quencher + 10.8 mL water.

4.5 Add 8 mL 1x Europium Quencher to a centrifuge tube.

4.6 After 15 minutes, add 2 mL of 1X Europium Quencher to stop the reaction, and mix by inverting several times.

4.7 Transfer the quenched reaction to the centrifuge tube containing 8 mL 1x Europium Quencher, invert to mix and incubate for 5 minutes at room temperature to quench the reaction.

4.8 Spin at 17,100 x *g* for 15 minutes. Remove ~8.5 mL of the supernatant and without resuspending the pellet spin for 1 more minute at 17,100 x *g*. Remove the rest of the supernatant.

4.9 Gently tap the pellet first and then add 400 µL of Resuspension Buffer. Thoroughly resuspend the pellet by pipetting up and down for at least 90 seconds.

4.10 You now have 400 µL 1 % conjugate.

4.11 Dilute the conjugate as required for your application.

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

5. Conjugated antibody storage:

Storage at 4°C is recommended for any conjugate. 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.

6. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)