ab269965 Antibody Concentration and Clean Up Kit for Latex & Europium

A product of Expedeon, an Abcam company

Applicable to Expedeon product codes 860-0005, 860-0010.

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Antibody Concentration and Clean Up Kit for Latex & Europium datasheet:

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For the Conjugation of Antibodies or Proteins to Latex and Europium nanoparticles.

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

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1. Overview

Some buffer components interfere with the conjugation reaction of the Latex and Europium Conjugation kits, reducing or entirely preventing conjugation to the latex nanoparticles. This Antibody Concentration and Clean Up Kit for Latex & Europium (ab269965) removes small molecule buffer components from the antibody or protein prior to use in the conjugation reaction.

This kit utilizes a simple spin column to clean up the antibody by buffer exchanging to remove the unwanted buffer components. The antibody is quickly and easily concentrated and then diluted with one of the Conjugation Kit Reaction Buffers for Nanoparticles. This step is repeated several times. This exchanges the antibody into a buffer perfect for the conjugation reaction and reduces the concentration of the initial buffer by several orders of magnitude so interference does not occur.

There are two Conjugation Kit Reaction Buffers, A and B, both of which are provided in this kit. One Spin Cartridge/Collecting Tube Assembly can clean up an antibody into one of the two buffers. To clean up the antibody into both Reaction Buffers, two Spin Cartridge/Collecting Tube Assemblies must be used.

The optimal buffer for conjugation varies for different antibodies, which is why both are provided. An antibody cleaned up into 1x Reaction Buffer A, but conjugated in 1x Reaction Buffer B (and vice versa), will have a conjugation efficiency of 50 – 100 % compared to both cleaning up and conjugating in the optimal buffer. For optimal conjugation efficiency we therefore recommend determining the optimal 1x Reaction Buffer then ensuring antibody for future conjugations is cleaned up into the optimal buffer. For quick scouting or 'proof of principle' experiments this may not be necessary.

If needed the Antibody Concentration and Clean Up Kit for Latex & Europium can also be used to concentrate the antibody by recovering the antibody in a smaller volume than the volume initially added to the Spin Cartridge.

2. Materials Supplied and Storage

Store kit at 4°C immediately on receipt and check below for storage for individual components. 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
Spin Cartridge/Collecting Tube Assembly	4 Tubes	4°C
20X Reaction Buffer A	1 vial	4°C
20X Reaction Buffer B	1 vial	4°C

Reagents are ready to use as supplied.

3. Technical Considerations

3.1 Buffer component removal:

The Antibody Concentration and Clean Up Kit for Latex & Europium (ab269965) can remove the following buffer component:

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

3.2 Concentration Determination:

As antibody/protein recovery from a column such as this is 60 – 100%, the concentration of the antibody/protein should be determined after clean up.

Ideally, this should be done using the absorbance of light at 280 nm. For an IgG antibody an A280 of 1.0 AU for a 1 cm light path is equivalent to an antibody concentration of 0.714 mg/mlmL. For other proteins the Beer-Lambert law can be used if the Extinction Coefficient is known.

If a Bradford-type reagent is used it is important to use a standard curve that is as similar to the antibody/protein as possible. You should use an IgG standard curve for an antibody of unknown concentration as the absorbance generated by this type of reagent is dependent on the protein used. If you use a BSA standard curve to determine the protein concentration of an IgG solution it will result in a 2.3-fold underestimation of the IgG concentration.

If it is not possible to determine the protein concentration, assume the protein recovery to be 80% and try the conjugation at different antibody/protein concentrations in case the estimate is wrong.

4. Assay Procedure

4.1 Dilute the selected Reaction Buffer to 1X. 5 mL is sufficient for one Spin Cartridge/Collecting Tube Assembly clean up. To make 5 mL, take 250 µL 20X Reaction Buffer A or B, add to 4750 µL deionized water and mix.

4.2 Add antibody/protein to the Spin Cartridge, top up to 500 µL with 1X Reaction Buffer and mix by pipetting.

4.3 Place Spin Cartridge/Collecting Tube Assembly in centrifuge with the printed text on the Spin Cartridge facing out, and spin for 2 – 5 minutes at 15,000 x *g* until only 100 – 150 µL is left in the Spin Cartridge.

4.4 Discard the flow through from the Collecting Tube.

4.5 Add 400 µL 1X Conjugation Buffer to the Spin Cartridge, gently mix by pipetting with a P200 and spin for 2- 5 minutes at 15,000 x *g* (ensuring text on Spin Cartridge faces out) until only 100 – 150 µL is left in the Spin Cartridge.

4.6 Discard the flow through from the Collecting Tube.

4.7 Repeat Steps 5 and 6 four more times.

4.8 If concentrating the antibody/protein, ensure the final volume in the Spin Cartridge is less than the initial volume added.

4.9 Gently mix by pipetting with a P200, then remove the sample from the Spin Cartridge.

*Δ **Note**: It is advisable not to spin the antibody dry as reconstitution of the antibody will be difficult and significant antibody loss and degradation may occur.*

*Δ **Note**: Spin times will vary depending on buffer composition and volume, as well as centrifuge speed and the characteristics of the antibody/protein itself.*

5. Antibody storage:

1x Reaction Buffers A and B are not optimal storage buffers for antibodies/proteins as they were developed for efficient conjugation for both Latex and Europium Conjugation Kits. We advise you to clean up into the Reaction Buffer and perform the conjugation straight away. Where this is not possible, short term storage at 4°C for up to a week, or longer storage frozen at -80°C, may be possible. Successful storage and sensitivity to freeze-thawing in these buffers will be dependent on the characteristics of the antibody/protein so must be determined experimentally.

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