PROTOCOL

Antibody Concentration & Clean Up Kit for Latex and Europium

For use with Latex Conjugation Kits and Europium Conjugation Kits

Applicable to: 1020-0040 for Latex & Europium Release 1. © EXPEDEON. 26/03/2018

INTRODUCTION

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 & Clean Up Kit for Latex and Europium removes small molecule buffer components from the antibody or protein prior to use in the conjugation reaction. To remove unwanted proteins such as BSA or gelatin from the antibody, please see our range of AbPure™ kits.

The Antibody Concentration & Clean Up Kit for Latex and Europium 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 Latex and Europium. 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 (Figure 1) 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.

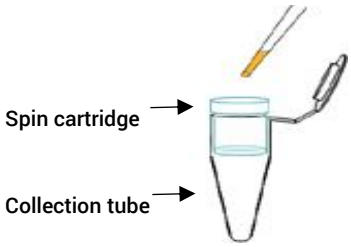

Figure 1. Spin Cartridge/Collecting Tube Assembly

This simple qualitative lateral flow assay does not require any specialized or costly equipment. The signal intensities can be qualitatively analyzed using the supplied scoring card or, for a quantitative detection, a LFA reader can be used.
If needed the Antibody Concentration & Clean Up Kit for Latex and 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.

KIT CONTENTS

- 4 Spin Cartridge/Collecting Tube Assemblies
- 1 vial 20x Reaction Buffer A
- 1 vial 20x Reaction Buffer B

SHIPPING CONDITIONS

The kit is shipped at ambient temperature. Store the kit at 4°C upon receipt.

INSTRUCTIONS

1. Dilute the selected Reaction Buffer to 1x. 5ml is sufficient for one Spin Cartridge/Collecting Tube Assembly clean up. To make 5 ml, take 250µl 20x Reaction Buffer A or B, add to 4750µl deionised water and mix.
2. Add antibody/protein to the Spin Cartridge, top up to 500µl with 1x Reaction Buffer and mix by pipetting.
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 15,000g until only 100 – 150µl is left in the Spin Cartridge.
4. Discard the flow through from the Collecting Tube.
5. Add 400µl 1x Conjugation Buffer to the Spin Cartridge, gently mix by pipetting with a P200 and spin for 2- 5 minutes at 15,000g (ensuring text on Spin Cartridge faces out) until only 100 – 150µl is left in the Spin Cartridge.
6. Discard the flow through from the Collecting Tube.
7. Repeat Steps 5 and 6 four more times.
8. If concentrating the antibody/protein, ensure the final volume in the Spin Cartridge is less than the initial volume added.
9. Gently mix by pipetting with a P200, then remove the sample from the Spin Cartridge.

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

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/ml. 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.

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.

BUFFER COMPONENTS REMOVED

Please see the table below for details of the buffer components that this kit can remove.

Buffer components	Latex and 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] If the antibody contains other proteins such as BSA or Gelatin this kit cannot be used and we recommend instead using our range of AbPure™ kits.

RELATED PRODUCTS

Description	Prod. Code
Europium Conjugation Kit – Midi Vial Conjugation	1200-0120
Latex Conjugation Kit – Midi Vial Conjugation	1000-0120 1002-0120 1004-0120
AbPure™ Magnetic Purification System	265-0200
AbPure™ Antibody Purification System	260-0010

TECHNICAL SUPPORT

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