PROTOCOL

Latex Conjugation Kit – Midi Vial Conjugation

Applicable to: **Blue Latex**: 1000-0120, **Red Latex**: 1002-0120, **Black Latex**: 1004-0120.

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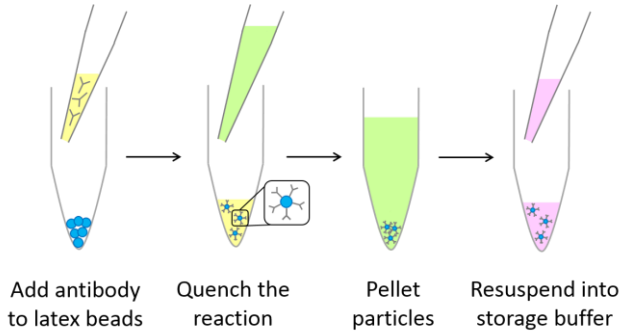
INTRODUCTION

The Latex Conjugation Kit allows antibodies or proteins to be conjugated to our high quality 400 nm latex nanoparticles quickly and easily. The latex nanoparticles are designed for ease of use and have much improved handling compared with traditional latex. This has been achieved by specially treating the nanoparticles. The conjugation is covalent, and requires less antibody than traditional passive and covalent latex conjugation.

The 400 nm blue, red or black latex nanoparticles 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 latex nanoparticles, then centrifuge to buffer exchange (Figure 1).

Figure 1. Latex Conjugation



The resulting covalent conjugates can be easily resuspended without the need for harsh methods such as sonication or vortexing, unlike traditional conjugation procedures which are prone to aggregation. This is due to the properties of the surface treatment which makes the particles resistant to aggregation.

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 would suggest that the antibody to be conjugated should be in 10 – 50 mM MES, HEPES or MOPS at pH 6 – 7 (with no other components e.g. salt or azide) before conjugation. See "Buffer Considerations" for more details and advice if your antibody is in another buffer.

KIT CONTENTS

- 1 Midi vial of 400 nm Blue, Red or Black Latex
  Each Midi vial gives sufficient conjugate for ~500 lateral flow tests
- 1 vial of 1x Reaction Buffer A
- 1 vial of 1x Reaction Buffer B
- 1 vial of 10x Quencher
- 1 vial of Resuspension Buffer

SHIPPING CONDITIONS

The kit is shipped at ambient temperature in a tamper-evident polypropylene container. Please 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. 450 µl will be sufficient to conjugate one 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. You may also wish to examine the effect of varying the amount of antibody, by testing different antibody dilutions. The 10 reaction Mini kit is designed for such optimisation experiments.*

3. Add 400 µl of the 0.1 mg/ml antibody to the Midi vial, and reconstitute the Latex nanoparticles by gently and thoroughly pipetting up and down. Incubate the reaction for exactly 15 minutes at room temperature.

4. Dilute the 10x Quencher with deionised water. You need exactly 10 ml, so we advise you to make 12 ml 1x Quencher: 1.2 ml 10x Quencher + 10.8 ml water.

5. Add 8 ml 1x Quencher to a centrifuge tube.

6. After the 15 minute incubation add 2 ml 1x Quencher to the vial to stop the reaction, and mix by inverting several times.

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

8. Spin at 10,000 rpm for 15 minutes. Remove ~8.5 ml of the supernatant and without resuspending the pellet spin for 1 more minute at 10,000 rpm. Remove the rest of the supernatant.

9. Gently resuspend the pellet in 400 µl Resuspension Buffer or a storage buffer of your choice.

   NOTE: *For increased stability of the liquid conjugate we advise adding 0.1% BSA (final concentration) to the Resuspension Buffer just prior to use. e.g. add 25 µl 2% BSA diluted in deionised water to 500 µl Resuspension Buffer.*

10. You now have 400 µl 1% conjugate. Dilute further as required for 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 nanoparticles (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 (see buffer considerations below). 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 400µl 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 COMPONENTS REMOVED

There are a number of common buffer components that have a substantial negative effect on the conjugation efficiency.
This decreases the amount of antibody that will be coupled to each nanoparticle, so reduces the signal from and sensitivity of the conjugated latex.

To prevent this we advise conjugating only from stock antibodies that are at least 1 mg/ml in 10 – 50 mM MES, HEPES or MOPS at pH 6 – 7 (with no other components e.g. salt or azide).

These are not common antibody storage buffers, so we have developed the Antibody Concentration & Clean Up Kit for Latex and Europium for use with antibodies stored in other buffers and therefore not compatible with this kit.
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 for a list of compatible and incompatible buffer components for this 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, however our AbPure™ kits can be used to achieve this.

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 to be conjugated contains other proteins such as BSA or Gelatin we recommend using our range of AbPure™ kits to remove such unwanted proteins

TECHNICAL SUPPORT

For technical enquiries get in touch with our technical support team at: www.expedeon.com/contact/

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