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ab269893 Latex Conjugation Kit – 400 nm Red

A product of Expedeon, an Abcam company

Applicable to Expedeon product codes 1002-0040, 1002-0100, 1002-0120.

View ab269893
Latex Conjugation Kit – 400 nm Red datasheet:
www.abcam.com/ab269893
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For the Conjugation of Antibodies or Proteins to Latex nanoparticles.

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

Table of Contents

1. Overview

Latex Conjugation Kit – 400 nm Red (ab269893) 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 Red 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.

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).

2. Materials Supplied and Storage

The kit is shipped at ambient temperature in a tamper-evident polypropylene container. 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	
400 nm Red Latex 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 Quencher	1 vial	1 vial	1 vial	+4°C or-20°C
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 and 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 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 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 (ab269965) to remove interfering buffer components (see "Buffer considerations before conjugation" section 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 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:

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 (ab269965) 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.

Recommended pre-conjugation buffer components and conditions:

Buffer components		Antibody Concentration & Clean Up Kit for Latex and Europium (ab269889) can remove
pH 6 - 7	Yes	Yes
pH < 6 and > 7	No	Yes
Amine free buffer (≤50 mM) *(e.g. MES, MOPS, HEPES)*	Yes	Yes
Amine free buffer (≥50 mM) *(e.g. MES, MOPS, HEPES)*	No	Yes
Salt	No	Yes
Sodium Azide	No	Yes
Sugars	Yes	Yes
Glycerol	Yes	Yes
Thiomersal	No	Yes
Thimerosal	No	Yes
Merthiolate	No	Yes
BSA	No	No[1]
Gelatin	No	No[1]
Tris	No	Yes
Glycine	No	Yes
Carboxylic acids *(e.g. EDTA, Citrate)*	No	Yes
Nucleophilic components *(Primary amines e.g. amino acids or ethanolamine and thiols e.g. mercaptoethanol or DTT)*	No	Yes

[1] If the antibody to be conjugated contains other proteins such as BSA or Gelatin we recommend using on of our Antibody Purification Kits compatible with Nanoparticle Conjugation Kits.

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 400 nm Red Latex 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 400 nm Red Latex vial and reconstitute the Latex nanoparticles by gently and thoroughly pipetting up and down. Incubate the reaction for exactly 15 minutes at room temperature.

4.4 Dilute sufficient 10x Quencher with deionised water. For one 400 nm Red Latex vial you need exactly 1 mL so we advise you to make 1.2 mL 1x Quencher per vial i.e. 120 µL 10x Quencher + 1080 µL water.

4.5 After 15 minutes, add 1 mL of 1X 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 10,000 rpm for 9 minutes. Remove ~850 µL of the supernatant and without resuspending the pellet spin for 1 more minute at 10,000 rpm. Remove the rest of the supernatant.

4.7 Gently resuspend the pellet in 40 µL Resuspension Buffer or a storage buffer of your choice.

*Δ **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 2.5 µL 2% BSA diluted in deionised water to 50 µL Resuspension Buffer.*

4.8 You now have 40 µL 1 % conjugate. Dilute the conjugate as required for 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 400 nm Red Latex 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 x 4 µg kit of ab269893 is designed for such optimisation experiments.*

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

4.4 Dilute sufficient 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.

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

4.6 After 15 minutes, add 2 mL of 1X 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 Quencher, invert to mix and incubate for 5 minutes at room temperature to quench the reaction.

4.8 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.

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

*Δ **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.*

4.10 You now have 400 µL 1 % conjugate. Dilute the conjugate as required for 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.

Technical Support

For all technical or commercial enquiries please go to:

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