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ab269890 Magnetic Conjugation Kit

A product of Expedeon, an Abcam company

Applicable to Expedeon product codes: 1300-0005, 1300-0010, 1300-0015

View ab269890
Magnetic Conjugation Kit datasheet:
www.abcam.com/ab269890
(use www.abcam.cn/ab269890 for China, or www.abcam.co.jp/ab269890 for Japan)

For the conjugation of antibodies or proteins to magnetic particles.

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

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1. Overview

Magnetic Conjugation Kit (ab269890) allows antibodies to be covalently attached to high quality 500 nm (0.5 µm) magnetic particles quickly and easily using one-step chemistry instead of the traditional EDC/NHS ester approaches. The conjugation reaction is initiated simply by reconstituting the dry mixture with your antibody which becomes attached (via lysine residues) to the magnetic particles. It takes 30 seconds to set up the conjugation and the hands-on time for the procedure is about 3 minutes. The covalent conjugate is ready to use within 1 hour.

Magnetic conjugates with covalent attachment of antibodies are ideal for immunoprecipitation experiments and provide a more stable alternative to non-covalent attachment of antibodies using protein A or protein G resin.

2. Materials Supplied and Storage

Store kit at -20°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	3 x 20 µg	10 x 20 µg	1 x 200 ug	Storage temperature
Magnetic particles vials	3 vials	10 vials	1 vial	-20°C
Mag Antibody Diluent	1 vial	1 vial	1 vial	-20°C
Mag Wash Buffer	1 vial	1 vial	1 vial	-20°C
Mag Quencher	1 vial	1 vial	1 vial	-20°C
Mag Storage Buffer	1 bottle	1 bottle	1 bottle	-20°C

3. Technical Considerations:

Buffer Considerations:

3.1 The purified antibody to be labeled should ideally be in 10 – 50 mM amine-free buffer (e.g. MES, MOPS, HEPES), pH range 6 to 9.

PBS affects the conjugation reaction to a certain extent when added neat to the conjugation vial. The interference is overcome if PBS is 1:2 diluted in the reaction mixture, therefore the antibody should be at least twice as concentrated as the requirement. As an example, an antibody in PBS needs to be ≥0.8 mg/mL to conjugate 20 µg at 0.4 mg/mL. Most other buffers whose pH is comprised between 6.0 and 9.0 are compatible with the reaction.

Buffer components	
pH	6 – 9
Amine free buffer ≤100 mM (e.g. MES, MOPS, CHES)	Yes
Sugars	Yes
Glycerol	Yes
PBS	No
Salt	No
Thiomersal / Thimerosal	No
Merthiolate	No
Sodium Azide (>0.10%)	No
BSA	No
Gelatin	No
Tris	No
Glycine	No
Nucleophilic components (Primary amines e.g. amino acids or ethanolamine and thiols e.g. mercaptoethanol or DTT)	No

*Δ **Note:** If the antibody is not in a suitable buffer for conjugation, please consult our Antibody Purification Kits.*

3.2 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 particles (surface area) and on the application; you may need to conjugate different amounts of antibody to optimize your assay.

We recommend adding 20 µg per vial for the 20 µg reaction scale in the first instance, but a range of 5 µg to 25 µg has

been shown to work with reference antibodies, ideally in a volume of 50 µL (i.e. concentration range is 0.1- 0.5 mg/mL). The same considerations apply to the 200 µg reaction scale, but the antibody amount needs to be increased by a factor of 10.
The volume of antibody will affect the rate of reaction; please contact us if your antibody is too diluted to use.

4. Assay Procedure

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

4.2 Dilute your stock antibody to 0.4 mg/mL with Mag Antibody Diluent.

4.3 We recommend to add 50 µL of the 0.4 mg/mL antibody to the 20 µg vial, or 500 µL to the 200 µg vial, and to reconstitute the magnetic particles by gently and thoroughly pipetting up and down. Transfer the magnetic particles from the 20 µg vial into a microcentrifuge tube. Split the magnetic particles from the 200 µg vial into two microcentrifuge tubes (250 µL each). Incubate the reaction for 30 minutes at room temperature with constant mixing (e.g. on a spiramix).

4.4 Place the tube on a magnetic stand for 5-10 seconds to collect the particles and remove the supernatant*.

4.5 Add 200 µL of Mag Wash Buffer to the tube for the 20 µg reaction scale or 1 mL to each tube for the 200 µg reaction scale, and mix thoroughly for 15 seconds. Place the tube on a magnetic stand for 5-10 seconds to collect the particles and discard the supernatant.

*Δ **Note**: the Mag Wash Buffer has a low pH, proceed quickly with the washing steps as indicated.*

4.6 Repeat the previous washing step.

4.7 Add 200 µL of Mag Quencher to the tube for the 20 µg reaction scale or 1 mL to each tube for the 200 µg reaction scale and mix thoroughly. For extra blocking, add BSA to 0.1% final concentration. Leave the reaction to quench for 30

minutes at room temperature with constant mixing. Place the tube on a magnetic stand for 5-10 seconds to collect the particles and discard the supernatant.

4.8 Add 200 µL of Mag Storage Buffer to the tube for the 20 µg reaction scale or 1 mL to each tube for the 200 µg reaction scale and mix thoroughly. Place the tube on a magnetic stand for 5-10 seconds to collect the particles and discard supernatant.

4.9 Repeat the previous washing step twice.

4.10 Add 25 µL of Mag Storage Buffer to the tube for the 20 µg reaction scale or 125 µL to each tube for the 200 µg reaction scale and mix thoroughly. Pool the tubes for the 200 µg reaction scale.

4.11 You now have 25 µL/250 µL 1% conjugate. Dilute further as required for your application. Optionally add preservative to the conjugate.

*The amount of antibody bound to the particles can be estimated analysing the supernatant with a Bradford protein assay and calculating the difference from the initial amount.

4.12 **Storage of conjugate:** Storage at 4°C is recommended for any conjugate. The Mag Storage Buffer added at the end of the conjugation reaction is a good storage buffer. **Do not store the conjugate at -20°C.**

5. FAQs

1. How many magnetic particles are in each kit?

A small size vial provides 25 µL 1% conjugated particles, and a big size vial provides 250 µL 1% conjugate. The amount of conjugate needed is very much dependent on the downstream application.

2. Can antibodies from different species be used?

Yes. We have tested antibodies from a variety of species including mouse and goat.

3. Is the kit suitable for conjugating small molecules including analytes?

The Magnetic Conjugation Kit has been optimized for antibodies, but it may be possible to conjugate small molecules with primary amine functional groups. Please contact our Scientific Support Team for more help.

4. What can I do if my antibody is not in a compatible buffer?

PBS affects the conjugation reaction to a certain extent when added neat to the 20 µg vial. The interference is overcome if PBS is diluted 1:2 in the reaction mixture, therefore the antibody should be at least twice as concentrated as the requirement. The conjugation reaction is compatible with most other buffers that have a pH between 7 and 9, and which have a lower concentration than 100 mM. Sodium azide at a concentration up to 0.1% in the reaction mix can be tolerated. The buffer shouldn't contain amines as these will interfere with the reaction. Should your buffer not be compatible with the Magnetic Conjugation Kit, you can use the Antibody Concentration and Clean-Up kit – Nanoparticles (ab204911) to exchange the antibody buffer.

5. What can I do if my antibody has non-antibody protein components or is not purified?

To remove interfering proteins before conjugation, or to purify your antibody from ascites fluid, serum or tissue culture supernatant, please see our Antibody Purification Kits.

6. What can I do if my antibody is 0.4 mg/mL or less?

If your antibody buffer is compatible with the conjugation reaction, you can add it without further dilution into the 20 µg vial. We recommend adding 50 µL of 0.4 mg/mL concentrated antibody which corresponds to 20 µg per vial, however an antibody concentration range of 0.2-1 mg/mL can be explored. The volume of antibody will affect the rate of reaction, so we advise to maintain the volume at 50 µL. Please contact Scientific Support Team if your antibody is too dilute to use.

7. How can I prevent unspecific binding?

It is beneficial to add a slight excess of antibody to the magnetic particles to fully cover the surface. The quencher provided blocks any reactive groups that are left at the end of the conjugation, however BSA can be spiked with the quencher to further prevent non-specific binding.

8. How do I know that the conjugation has worked?

The best way to test your conjugate is to use it in your assay. However you can confirm and estimate the amount of antibody bound to the beads by Bradford protein assay. Analyze your starting antibody mix, if free from any other protein, and the supernatant containing the unbound antibody by preparing a standard curve using a protein that has a similar weight (ideally the same protein used for the conjugation). Calculate the difference between the loaded protein and the unbound protein to estimate the coupled antibody.

Technical Support

Austria
wissenschaftlicherdienst@abcam.com | 019-288-259
France
supportscientifique@abcam.com | 01.46.94.62.96
Germany
wissenschaftlicherdienst@abcam.com | 030-896-779-154
Spain
soportecientifico@abcam.com | 91-114-65-60

Switzerland
technical@abcam.com
Deutsch: 043-501-64-24 | Français: 061-500-05-30
UK, EU and ROW
technical@abcam.com | +44(0)1223-696000

Canada
ca.technical@abcam.com | 877-749-8807
US and Latin America
us.technical@abcam.com | 888-772-2226

Asia Pacific
hk.technical@abcam.com | (852) 2603-6823
China
cn.technical@abcam.com | 400 921 0189 | +86 21 2070 0500

Japan
technical@abcam.co.jp | +81-(0)3-6231-0940
Singapore
sg.technical@abcam.com | 800 188-5244

Australia
au.technical@abcam.com | +61-(0)3-8652-1450
New Zealand
nz.technical@abc.com | +64-(0)9-909-7829