Version 6b

ab102778 Antibody Concentration And Clean-up Kit Protocol

A product of Expedeon, an Abcam company

Applicable to Expedeon product codes 861-0005, 861-0010.

View ab102778
Antibody Concentration And Clean-up Kit datasheet:
www.abcam.com/ab102778
(use www.abcam.cn/ab102778 for China, or www.abcam.co.jp/ab102778 for Japan)

For preparing antibodies for conjugation.

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

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1. Overview

Antibodies are sometimes only available at low concentrations and often contain low molecular weight substances that interfere in labeling reactions with enzymes, biotin, streptavidin and fluorophores. The Antibody Concentration And Clean-up Kit allows for the quick and easy concentration of up to 3 different antibodies and proteins. The kit can also be used to reduce the concentration of many unwanted additives often found in antibody formulations such as azide, glycine or Tris.

The Antibody Concentration And Clean-up Kit method utilizes a simple spin column to easily and quickly remove excess buffer from the antibody thereby providing a more concentrated antibody solution. The Antibody Concentration And Clean-up Kit also allows the experimenter to perform a simple buffer exchange to transfer the antibody into a more favorable buffer for conjugation.

Antibodies concentrated using the Antibody Concentration And Clean-up Kit are fully compatible with our Lightning-Link® Antibody Conjugation kits and our Oligonucleotide Conjugation Kit.

2. Materials Supplied and Storage

Store at +4°C upon receipt.

Item	3 x Test	Storage temperature
Conjugation Buffer	1 vial	+4°C
Spin cartridge/collecting tube assembly (up to 0.5ml each)	3 cartridges (up to 0.5 mL each)	+4°C

Reagents are ready to use as supplied.

3. Assay Procedure

For buffer exchange, proceed directly to section 3.2.

3.1 For Concentration of Antibody Solution:

1. Add up to 0.5 mL antibody to spin cartridge.
2. Spin for 1 to 3 minutes in a microfuge at a recommended max speed of 15,000 x *g* to reduce the buffer volume in the spin cartridge to between 50 and 100 µL.
3. Repeat steps 1 to 2 as many times as is necessary to process the entire antibody to the desired concentration. It may be necessary to discard the excess buffer collected in the collection tube between spins.
4. Recover the concentrated antibody from the spin cartridge.

*Δ **Note**: It is advisable not spin the antibody dry as reconstitution of the antibody will be difficult and significant antibody loss and degradation may occur. Minimum volume advised for recovery is 50 µL.*

*Δ **Note**: Other proteins present in the buffer such as BSA will also be concentrated using this method. To remove unwanted proteins, see our selection of purification kits.*
*Δ **Note**: Spin times will vary depending on buffer composition and volume as well as centrifuge speed.*

3.2 For Buffer Exchange Using Spin Column Assembly:

1. Add up to 0.5 mL antibody to spin cartridge.
2. Spin for 1 to 3 minutes in a microfuge at a recommended maximum speed of 15,000 x *g* to reduce the buffer volume to 100 µL.
3. Discard the excess liquid in collection tube.
4. Add 400 µL conjugation buffer to the antibody in the spin cartridge.
5. Spin for 1 to 3 minutes in a microfuge at a recommended maximum speed of 15,000 x *g* to reduce buffer volume to 100 µL.
6. Discard the excess liquid in collection tube.
7. Repeat steps 2.4 to 2.6 at least 5 times to exchange antibody buffer.
8. Recover antibody from the spin cartridge.

*Δ **Note**: Each cycle leads to a reduction in the concentration of low molecular weight substances (smaller than 10 kDa). However, the concentration of proteins such as BSA (66.5 kDa) will be unchanged. To remove unwanted proteins, see our selection of purification kits.*

*Δ **Note**: The exchange process is more efficient if the volume is reduced to 50 µL instead of 100 µL at each cycle.*

*Δ **Note**: If the antibody requires a buffer exchange as well as being concentrated, it can be recovered in less volume at the end of the last buffer exchange step. Minimum volume advised for recovery is 50 µL.*

*Δ **Note**: Spin times will vary depending on buffer composition and volume as well as centrifuge speed.*

4. Test for protein concentration

Wherever possible protein values should be determined using an absorbance at 280 nm.

For an IgG using a 1 cm light path an OD280 of 1.0 is equivalent to an antibody concentration of 0.714 mg/mL.

When using Bradford-type reagents it is important to use an IgG standard curve. The absorbance generated by this type of reagent is dependent on the protein used. For example, using a BSA standard curve to determine the protein concentration of an IgG solution will result in a 2.3-fold under-estimate of the IgG concentration.

5. Antibody Storage

Store at +4°C. Other storage conditions (e.g. frozen at -70°C) may also be satisfactory). The sensitivity of any particular antibody to freeze-thaw should be determined by experimentation on small aliquots.

Technical Support

For all technical or commercial enquiries please go to:
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