PROTOCOL

AbSelect™ Antibody Concentration & Clean-up Kit

Applicable to: 861-0010 3 columns Release 1.© EXPEDEON. 21/02/2018

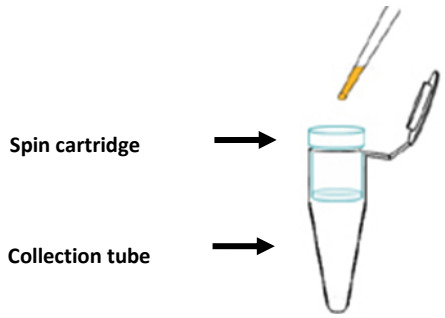
INTRODUCTION

Antibodies are sometimes only available at low concentrations and often contain low molecular weight substances that interfere in labeling reactions with enzymes, fluorophores, biotin and streptavidin. The AbSelect™ Antibody Concentration and Clean-Up Kit allows for the quick and easy concentration of 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 AbSelect™ Concentration and Clean-Up Kit utilizes a simple spin column to easily and quickly remove excess buffer from the antibody thereby providing a more concentrated antibody solution.

The AbSelect™ 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.

Figure 1. Spin Cartridge/collecting tube assembly



KIT CONTENTS

- 3 spin cartridge/collecting tube assemblies
- 1 bottle of AbSelect™ conjugation buffer

INSTRUCTIONS

1. Concentration of antibody solution:
   - 1.1 Add antibody to spin cartridge.
   - 1.2 Spin for 1 to 3 minutes* in a microfuge at a recommended maximum speed of 15,000g to reduce the buffer volume in the spin cartridge to between 50 and 100µl.
   - 1.3 Repeat steps 1 and 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.
   - 1.4 Recover the concentrated antibody from the spin cartridge.

Notes: It is advisable not spin the antibody dry as reconstitution of the antibody will be difficult and significant antibody loss and degradation may occur.

Other proteins present in the buffer such as BSA will also be concentrated using this method. To remove unwanted proteins see our AbSelect kits described in section 4.

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

2. Buffer exchange using spin column assembly:
   - 2.1 Add up to 0.5ml antibody to spin cartridge.
   - 2.2 Spin for 1 to 3 minutes* in a microfuge at a recommended maximum speed of 15,000g to reduce the buffer volume to 100µl.
   - 2.3 Discard the excess liquid in collection tube.
   - 2.4 Add 400µl conjugation buffer to the antibody in the spin cartridge.
   - 2.5 Spin for 1 to 3 minutes* in a microfuge at a recommended maximum speed of 15,000g to reduce buffer volume to 100µl.
   - 2.6 Discard the excess liquid in collection tube.
   - 2.7 Repeat steps 4 to 6 as at least 5 times to exchange antibody buffer.
   - 2.8 Recover antibody from the spin cartridge.

Notes: Each cycle leads to a reduction in the concentration of low molecular weight substances. However, the concentration of proteins such as BSA will be unchanged. To remove unwanted proteins see our AbSelect™ kits described in the last section of this protocol.

The exchange process is more efficient if the volume is reduced to 50µl instead of 100µl at each cycle.

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

3. Test for protein:

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

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

SHIPPING CONDITIONS

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

STORAGE OF ANTIBODY

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.

RELATED PRODUCTS

Description	Prod. Code
AbSelect™ Antibody Purification Systems	860-0005 860-0010

The AbSelect™ Antibody Purification method involves the capture of the antibody on a resin and the removal of unwanted substances by a simple wash procedure, which is carried out in a standard microfuge. The purified antibody is then eluted and neutralized.

All components in each AbSelect kit are compatible with our range of Lightning-Link® one-step conjugation kits. This means that you can go directly from purification to labeling.

Please visit our website www.expedeon.com to view our range of species-optimized (Protein A, Protein G or mouse-specific) and source-optimized (antibody, TCS or serum / ascites fluid) AbSelect kits. Protein A Agarose and Protein G Agarose are also available separately.

TECHNICAL SUPPORT

For technical enquiries get in touch with our technical support team at: technical.enquiries@expedeon.com
For further information see our website: www.expedeon.com