Innova Biosciences
making science easier

AbPure™ Antibody Concentration & Clean Up Kit

For use with gold nanoparticles

Applicable to:

262-0010 (3 columns)

Release 3 27/01/2015

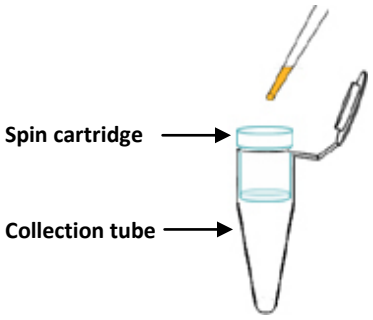
Introduction

Commercially available antibodies often contain substances (e.g. salt, citrate and azide) that interfere in conjugation reactions of InnovaCoat® Gold. The AbPure™ Antibody Concentration and Clean Up Kit allows removal of those contaminants.

The AbPure™ Antibody 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 AbPure™ Antibody Concentration and Clean Up Kit also allows the experimenter to perform a simple buffer exchange to transfer the antibody into a more favourable buffer for InnovaCoat® Gold conjugation.

Figure 1 – Spin cartridge / collecting tube assembly.



Kit components

3 spin cartridge/collecting tube assemblies

1 bottle of AbPure™ 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 to spin the antibody dry as reconstitution of the antibody will be difficult and significant antibody loss and degradation may occur.

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

Please note other proteins present in the buffer such as BSA will also be concentrated using this method.

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 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 AbPure™ kits.

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.

For further information see our website
www.innovabiosciences.com

For technical enquiries get in touch with our technical support team at
technical.enquiries@innovabiosciences.com

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.