OriGene Technologies Inc.

9620 Medical Center Drive, Ste 200
Rockville, MD 20850
UNITED STATES
Phone: +1-858-888-7900
Fax: +1-858-888-7904
US-info@acris-antibodies.com

OriGene EU

Acris Antibodies GmbH
Schillerstr. 5
32052 Herford
GERMANY
Phone: +49-5221-34606-0
Fax: +49-5221-34606-11
info@acris-antibodies.com

AbSelect Antibody Purification System (1 Purification)

Catalog No.: AC047-001

Quantity: 1 set

Reaction: 1 Purification

Kit Contents:

AC047-001-R:	Lyophilised AbSelect Protein A Resin
AC047-001-B:	1 vial of 10x Binding Buffer
AC047-001-W:	1 vial of Wash Buffer
AC047-001-E:	1 vial of Elution Buffer
AC047-001-N:	1 vial of Neutralization Buffer
AC047-001-T1:	1 spin cartridge/collecting tube assemblies
AC047-001-T2:	4 additional collecting tubes

Background:

Substances (e.g. BSA, glycine, tris, and azide) that interfere in labeling reactions. The AbSelect Purification System quickly removes these contaminants. It can also be used to purify antibodies from crude samples such as ascites fluid or immune serum. The antibody to be purified or cleaned up ideally is in a volume of 0.1 ml to 0.5 ml. 20 to 500 µg of antibody can be purified in each run.

The method involves capturing the antibody on the AbSelect Protein A Resin. Protein A has a high affinity for the Fc regions of IgG molecules from a variety of species (see Appendix). Once the antibody has bound to the Protein A, unwanted substances can be removed by simply washing the resin. The antibody is then eluted and neutralized.

The AbSelect Antibody Purification System is fully compatible with both the Light-A-Link conjugation systems (available separately), which allow the purified antibody to be labeled with a hands-on time of under 30 seconds.

Principle of the Procedure:

Step 1: Reconstitute Protein A Resin **AC047-001-R** with Wash Buffer **AC047-001-W**. **Step 2:** Transfer to Spin cartridge **AC047-001-T1** and spin briefly. **Step 3:** Add antibody supplemented with 10x Binding Buffer **AC047-001-B** and incubate for 1 hour. **Step 4:** Wash Resin. **Step 5:** Elute and neutralize purified antibody. **Step 6:** Confirm antibody is in eluate using a test for protein.

Protocols:

1. Reconstitution of AbSelect Protein A Resin

Add 0.5ml of Wash Buffer **AC047-001-W** to the vial of Protein A Resin, mix by inversion for a few seconds and transfer to the Spin cartridge **AC047-001-T1** (Figure 1). Spin for 30 seconds in a microfuge.

2. Incubation of Sample with Resin

Add the appropriate amount of 10x Binding Buffer **AC047-001-B** to the antibody. For example, if the sample volume is 200 µl, add 20 µl of Binding Buffer. Pipette the sample into the spin cartridge and cap the tube. Incubate for 1 hour with agitation or periodic

shaking.

Note: The volume of antibody to be purified or cleaned up should be 0.1-0.5 ml, though larger volumes may be processed by first incubating the antibody sample with the Protein A Resin **AC047-001-R** in a larger vessel (e.g. 2ml eppendorf) prior to transferring to the spin cartridge.

3. Wash Procedure
Microfuge the spin cartridge assembly for 30 seconds to remove most of the non-bound protein. Add 0.5 ml of Wash Buffer **AC047-001-B** and spin again. Perform the wash procedure three times.

Note: Save the non-bound and wash fractions by transferring the material from the collecting tube after each spin to a set of eppendorfs (not supplied). Do not use the four (or twelve) collecting tubes **AC047-001-T2** supplied with the kit, as these have an extended hinge to accommodate the spin cartridge, and are required for the Elution step.

4. Elution
Transfer the cartridge to a clean collecting tube **AC047-001-T2**. Add 100 µl of Elution Buffer **AC047-001-E** and incubate for 2 minutes at room temperature with gentle agitation. Microfuge for 30 seconds. Remove the collecting tube (See Section 5) and add 25 µl Neutralization Buffer **AC047-001-N**.

Place the cartridge in a new collecting tube and add a further 100 µl of Elution Buffer **AC047-001-B** to the Protein A Resin. Incubate for 2 minutes at room temperature with gentle agitation. Spin and collect and neutralize as before.

Repeat the Elution procedure until all four clean collecting tubes have been used. The protein normally elutes in tubes 1 and 2 but you should confirm this using a test for protein (See Section 5) before pooling any of the tubes.

Pool the tubes with the most protein (this is normally two tubes; if more than two tubes are strongly positive it is possible that you have used too much sample in your protein assay.)

However, if your application does not require a high concentration of antibody you may choose to pool all tubes that contain protein, regardless of concentration.

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.

5. Test for Protein
Wherever possible protein values should be determined using an absorbance at 280nm.

When other methods are used such as BCA or Bradford protein assays, determinations should be performed before the addition of the Neutralization Buffer **AC047-001-N**. The Neutralization Buffer **AC047-001-N** contains components that can interfere with these reagents. The Neutralization Buffer should be added to the sample as soon as possible as the low pH of the Elution Buffer **AC047-001-E** can denature the antibody.

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.

Storage:

Storage of Kit: The kit is shipped at ambient temperature. Store the kit at 4°C upon receipt.
Shelf life: One year from Despatch.

Pictures:

Appendix: Protein A affinity for immunoglobulins.

Species	Ig	Binding strength
Rabbit	IgG	High
Human	IgG	High
Mouse	IgG_1	Medium/High
Mouse	IgG_{2a}	High
Mouse	IgG_{2b}	High
Mouse	IgG_3	Medium
Goat	IgG	Low
Sheep	IgG	Low
Rat	IgG	Low

Figure 1: Spin cartridge / collecting tube assembly.