ademtech

IG ADEM-KIT 04400

For total Ig purification

For research use only

INTRODUCTION

The kit is based on the use of the **Biomagnetic separation technology. The separation method is gentle and does not require the use of columns or centrifugation step.**

Biomagnetic separation technology is a simple technique based on the separation **of superparamagnetic beads** using a magnetic field. When added to a complex medium, the magnetic particles will bind to the target. This interaction is based on the specific affinity of the ligand on the surface of the beads. The resulting target-bead complex can be removed from the suspension using a magnet. The benefits of magnetic handling are easy washing, separation and concentration of the target without any need for centrifugation or columns.

Superparamagnetic beads exhibit magnetic properties only when placed within a magnetic field and show no residual magnetism when removed from this field.

IG ADEM KIT PRINCIPLE

Ig Adem Kit is designed for simple and rapid immunoglobulin purification from serum, ascites culture or hybridoma supernatants. The kit was developed to overcome the drawbacks of the commonly used purification methods. Obtaining pure antibodies by removing non relevant protein often present in high abundance is a critical step. Immunoglobulins purification is based on the ability of some proteins to bind to a ligand that contains a sulfone group close to a thioether group. This binding event is termed **thiophilic adsorption**. Salts that interact with water molecules, such as potassium and ammonium sulfate, promote this binding.

Magnetic beads bind IgG (subclasses), IgA, and IgM in the same proportion, present in starting material.

The purified Ig can be used in a variety of applications including: ELISA, Western Blotting, Immunoaffinity, Immunoprecipitation.

Species	IgG (mg/ml)	IgM (mg/ml)	IgA (mg/ml)
Human	7.5-22	0.2-2.8	0.5-3.4
Mousse	2-5	0.8-6.5	1-3.2
Rat	5-7	0.6-1	0.1-0.2
Rabbit	12-14.5	0.3-0.6	0.4-0.8
Goat	18-24	0.8-2	0.1-0.9
Sheep	18-24	0.8-1.8	0.1-1
Horse	11.5-21	1-0.3	
Ascites	0.5-5		
Cell supernatant	0.01-0.05		

Table 1: Typical Immunoglobulin Concentration Ranges

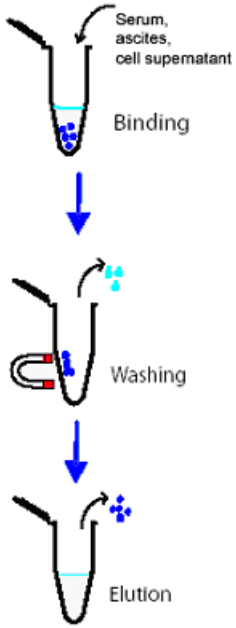
PRODUCT DESCRIPTION

The Kit contains all the components required for 15 Ig purification procedures in a prepackaged, easy to use format. The magnetic beads format is intended for single use. The kit is provided to perform 15 Ig purifications from up to 3.75 mg of IgG. Buffers are produced under asceptic conditions and are sold in an aqueous suspension containing 0.09% NaN_3.

	Amount	Component	Storage
R1	15 tubes	**Magnetic beads**	+ 2-8°C
R2	13 ml	**Binding Buffer**	+ 2-8°C
R3	100 ml	**Washing Buffer 3X**	+ 2-8°C
R4	30 ml	**Elution Buffer**	+ 2-8°C

Table 1: Components provided with the kit

PROTOCOL SUMMARY



INSTRUCTIONS FOR USE

The protocol is suitable for use with Ig from the following species: human and mouse. The kit may be applicable for the purification of Ig from other species; however, it is up to the individual researcher to determine the suitability for their particular use.

For best results we recommend adapting the binding step depending of the type of starting material and the immunoglobulin concentration in starting material.

After shipping, the cap can contain magnetic beads, place the tube on the magnet and invert the whole.

A) Binding step

1. Take a microtube of magnetic particles
2. Place the tube on the magnet for at least 15 seconds or until supernatant clearing and discard the supernatant.
3. Add 625µl of starting material.
4. Add 875µl of Binding Buffer.

Depending on the application and the starting material concentration, protocol can be customized. In order to obtain the correct binding condition, the following formula must be followed: **Volume of Binding Buffer required= volume of Ig Beads + volume of starting material**

5. Roll mix at room temperature at least 2 hours or overnight for maximum rate.

B) Washing step

1. Washing Buffer preparation

Dilute Washing Buffer 3-fold with distilled water. Divide it into conveniently sized aliquots according to your utilization and keep at 4 °C.

The step is only required for the first utilisation of the kit.

2. Place the tube on the magnet until pellet forming and discard the supernatant. Remove the tube from the magnet and add 1500µl Washing Buffer and mix by vortexing or inverting the tube.
3. Repeat 9 times steps 1& 2.
4. Monitor absorbance of the sample at 280nm to determine when all non-bound material is washed.

OD 280 nm of the last wash supernatant should be < 0.1 Contrariwise, repeat washing step.

C) Elution step

1. Place the tube on the magnet until until pellet forming and discard the supernatant. Remove the tube from the magnet.
2. Add 400µl Elution Buffer and mix by vortexing or inverting the tube. After 5 minutes with gentle mixing at room temperature place the tube on the magnet and keep the supernatant containing the most concentrated Ig Fraction.

3. Repeat twice step 2 using 400µl Elution Buffer. Keep the supernatant containing the second and third Ig Fraction.

D) Quantification of Ig

Measured the absorbance of each fraction at 280nm vs Elution Buffer.

To obtain the correct Ig concentration, the following formula must be followed:

Ig concentration= OD 280 nm /ε

ε IgG = 1.35 (1cm, 0.1%)

TROUBLESHOOTING

1. No antibody detected

Be sure not to have a sample devoid of Ig. Ensure by others means e.g. ELISA that the sample contains Ig.

2. Abundant non Ig protein recovered

Increase washing number and washing volume.

3. Purified Ig is colored :

Serum sample is hemolyzed.

Hemolysis of serum sample can be reduced or eliminated by performing any of the following procedures:
- Clot blood the same day of collection-do not clot blood overnight
- Collect blood in the presence of an anti-coagulant and centrifuge to remove the red blood cells
-Collect the blood with care to prevent hemolysis

ADDITIONAL MATERIAL REQUIRED

- Magnetic devices
  - Adem-Mag SV, 1.5 ml (# 20101)
  - Adem-Mag MODULO,12X1.5-2ml (#20105)
- Microtubes
- Rotation device
- Spectrophotometer

STORAGE / STABILITY

Properly stored kits are guaranteed for 6 month from the date of receipt. Note that the shipping is realized at room temperature which will not affect the stability of the product.

PRECAUTIONS

Precautions should be taken to prevent bacterial contamination. If cytotoxic preservatives are added they must be carefully removed before use by washing.

WARNINGS AND LIMITATIONS

For research use only. Not for use in human diagnostic or therapeutic procedures.

Sodium azide is toxic if ingested. **Avoid pipetting by mouth**. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. When disposing through plumbing drains, flush with large volumes of water to prevent azide buildup.

WARRANTY

The products are warranted to the original purchaser only to conform to the quality and contents stated on the vial and outer labels for duration of the stated shelf life.
Ademtech's obligation and the purchaser's exclusive remedy under this warranty is limited either to replacement, at Ademtech's expense, of any products which shall be defective in manufacture, and which shall be returned to Ademtech, transportation prepaid, or at Ademtech's option, refund of the purchase price.

Claims for merchandise damaged in transit must be submitted to the carrier.

Ordering Information

Product	Description	Code
Ig Adem-Kit	**15 Total Ig purification**	**04400**
Ig Adem kit	**30 Total Ig purification**	**04401**

Ademtech SA – Bioparc BioGalien - 27, allée Charles Darwin - 33600 PESSAC - FRANCE
www.ademtech@ademtech.com
Tel: +33557020
Fax +33557026