



NaCoMag Protein A MAGNETIC BEADS NM A1000

1. PRODUCT DESCRIPTION

1.1 Intended Use

NaCoMag Protein A coated magnetic beads are designed to capture immunoglobulins (Ig) for purification purposes or immunoassays.

1.2 Principle

Immunomagnetic protein isolation using NaCoMag NM A1000 provides a fast and reliable method for capturing Ig for purification or immunoassays. Ig can be isolated directly from ascites, serum, tissue culture supernatants or other samples.

An Ig-containing sample is added to a tube containing pre-washed NaCoMag NM A1000 beads. During a short incubation, the immunoglobulins will bind to NaCoMag NM A1000 via their Fc part. Place the test tube on a magnet to collect the NaCoMag NM A1000 - Ig complex at the tube wall, and discard the supernatant.

The purified and concentrated Ig can be eluted off in a small volume for downstream use.

The NaCoMag NM A1000 - Ig complex may also be used directly to immunoprecipitate a target antigen, or for immunodepletion. Add NaCoMag NM A1000 - Ig complex directly to a sample (cell lysate or other) containing your target antigen and incubate for antibody-antigen complex formation. Place the test tube on a magnet to collect the complex at the tube wall, and discard the supernatant. Resuspend the beads in a small volume for further use, or elute off your target protein directly e.g. in an acidic buffer or boil in a small volume of SDS-PAGE application buffer.

If your downstream application involves purification of your target protein, you might want to cross-link the Ig to the NM A1000 on the NaCoMag beads before immunoprecipitation to prevent co-elution of the Ig.

Human IgG1,2,4	Strong	Chicken IgY	No binding
Human IgD	No binding	Dog IgG	Strong
Human IgG3,A,E,M	Weak	Goat IgG1	Weak
Mouse IgG1	Weak	Goat IgG2	Strong
Mouse IgG2a,2b, 3	Strong	Guinea pig IgG	Strong
Mouse IgM	Weak	Hamster	Weak
Rat IgG1	Weak	Horse IgG	Weak
Rat IgG2a	No binding	Monkey IgG	Strong
Rat IgG2b	No binding	Porcine IgG	Strong
Rat IgG2c	Strong	Rabbit IgG	Strong
Bovine IgG1	Weak	Sheep IgG1	Weak
Bovine IgG2	Strong	Sheep IgG2	Strong

Table 1: Binding strength of protein A to different species of immunoglobulins (Ig) and their subclasses. Monoclonal antibodies will vary in their affinity towards protein A.

1.3 Description of Material NM A1000

NaCoMag NM A1000 beads are uniform, magnetisable superparamagnetic polystyrene beads covalently coated with a novel self-assembling coating protein combining a proprietary self-organizing proteinous anchor sequence with the Fc binding domain from Protein A, which has a high specificity for immunoglobulins and is thus suitable for the one-step capture of Ig. NaCoMag NM A1000 protein A coatings allow ideal, dense, non-denaturing and reproducible presentation of the IgG capturing domain ensuring unprecedented performance.

Diameter: 1.05 µm

The beads are supplied in TRIS buffered saline (TBS), pH 7.2, containing a stabilizing agent and 15 mM sodium azide (NaN₃).

Concentration: 5 mg/ ml

Binding capacity

Binding of Ig to protein A in solution is an equilibrium reaction.

The amount of Ig captured is dependent on the concentration of Ig in the starting sample. 200 µl (= 1 mg) NaCoMag NM A1000 beads will isolate approximately 54 µg IgG.

2. INSTRUCTIONS FOR USE

NaCoMag NM A1000 beads should be washed prior to use. Washed beads are resuspended in TBS or a basic buffer (e.g. 0.1 M glycine pH 9.0, or) to facilitate binding of Ig to beads. The pH in the sample containing Ig might be adjusted for the same reason using a basic 5 x stock solution. The use of polypropylene tubes is recommended for washing and Ig capture.

2.1 Washing Procedure

The washing procedure is facilitated by the use of a magnet.

1. Resuspend NaCoMag NM A1000 coated beads, thoroughly in the vial (e.g. by vortexing 1-2 minutes or rotating on a roller) to obtain a homogeneous suspension.
2. Transfer 200 µl of the solution containing NaCoMag NM A1000 beads to a test tube containing 800 µl buffer at room temperature.
3. Place the test tube on the magnet for one minute and pipette off the supernatant.
4. Remove the test tube from the magnet and add 1 - 2 ml buffer.
5. Repeat steps 3, 4 and 3.

Separate the beads during the washing steps using a vortex.

2.2 Ig Capture Procedure

1. Add 500 µl of a solution containing IgG (up to 400 µg) to the beads. If serum samples are present use 0.1 M glycine buffer pH 9 or a basic 5 x stock solution to adjust the pH to a basic range.
2. Incubate with slow tilt rotation mixing for 15 minutes at room temperature.
3. Place the test tube on the magnet for 1 - 2 minutes and pipette off the supernatant.
4. Remove the test tube from the magnet and add 1 ml washing buffer or 1 ml 0.5M NaCl.
5. Place the test tube on the magnet for 2 minutes and pipette off the supernatant.
6. If desired repeat steps 3, 4 and 5.

The purified Ig is now ready to be eluted off the beads or the bead NM A1000 - Ig complex can be used for immunoprecipitation - either by adding directly to a new sample containing the target protein, or by first cross-linking the Ig covalently to the NM A1000 beads.

2.3 Ig Elution Procedure

Eluting Ig off the beads is performed by lowering pH using 0.1 M glycine (pH 2.5-3) as the elution buffer. The degree of acidity needed depends on the species and Ig subclass.

1. Add an appropriate amount (up to 1000 µl) 0.1 M glycine (pH 2.5) to the NM A1000 bead - Ig complex with immobilised IgG.
2. Mix well by vortexing and incubate on a rotator for 10 minutes.
3. Place the test tube on a magnet and transfer the supernatant, containing purified Ig, to a clean tube. Immediately adjust the eluate to physiologic pH by adding alkaline buffer (e.g. 1M Tris pH 7.5-9).

3 Re-use of NaCoMag NM A1000 beads

For re-use after elution, the beads should be washed and stored in 0.1 M TBS buffer having azide added. If aggregation occurs add non-ionic detergent or use ultrasonication. Beads can be re-used up to 10 times.

4 Storage and Stability

This product is stable when stored unopened at 2-8°C. Store opened vials at 2-8°C and use care to avoid bacterial contamination.

Do not freeze the product.

Keep beads in liquid suspension during storage and all handling steps, as drying will result in reduced performance. Resuspend well before use.

FOR RESEARCH USE ONLY