IMMAGINA BioTECHNOLOGY s.r.l
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Via del Castel, 21 - Mattarello
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Instruction 016-01-003 MAGAR-cN™

MAGAR-cN™are available in the following pack size:

MAGAR-cN™	Crosslinked magnetic beads functionalized with Neutravidin™, 10% medium slurry, 1 ml

Note: 1 ml medium slurry is sufficient for 10-20 reactions according to the recommended immunoprecipitation protocol. 1 ml of 10% (v/v) medium slurry contains 100 µl of magnetic beads.

Purpose
MAGAR-cN™ is intended for research use only, and should not be used in any clinical or *in vitro* procedures for diagnostic purposes.
Application
MAGAR-cN™ contains magnetic beads that enable enrichment of proteins by immunoprecipitation. MAGAR-cN™ can be used for biomarker research.
Binding Capacity
>0.03 mmol biotinylated small molecule/ml of medium slurry
Storage Conditions
4 - 8°C, 20% ethanol
Stability
Highly stable in suspension
Dimension
40-60 micron
Storage temperature
+4°C to +8°C
Notes
DO NOT FREEZE

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Preparing buffers and samples - Recommended buffers

Note: Use high-purity water and chemicals for buffer preparation. Buffers are not provided by IMMAGINA.

Binding buffer	2M NaCl, 1mM EDTA, 10 mM Tris-HCl pH 7.5
Washing buffer 1 (mild)	50 mM Tris HCl, 150 mM NaCl
Washing buffer 2 (medium)*	50 mM Tris HCl, 150 mM NaCl, 0.01% Tween 20
Washing buffer 3 (strong)*	50 mM Tris HCl, 150 mM NaCl, 2 M urea
Incubation Buffer	50 mM Tris HCl, 150 mM NaCl, pH 7.5
Elution buffer	100°C for 10 min in the presence of 1% SDS or 1x Laemmli Sample Buffer **OR** 0.1 M glycine HCl, 2 M urea, pH 3

(*) see step 6 only

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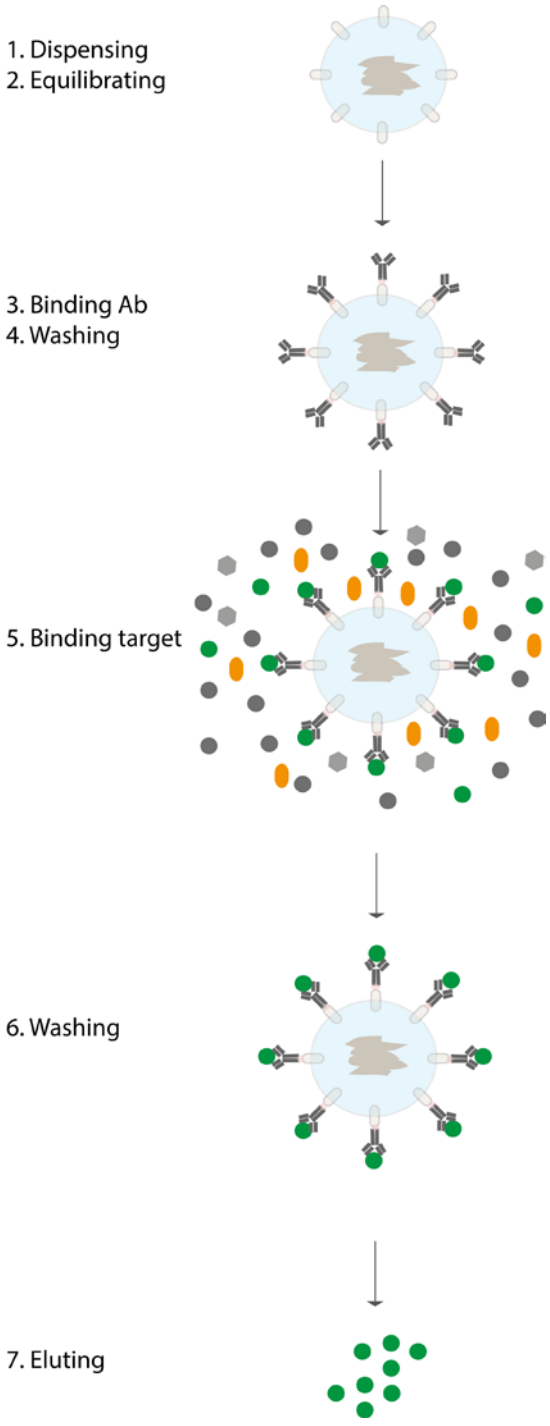

Scheme I

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Protocol for immunoprecipitation (Scheme I)

This protocol is suitable as a starting point for enrichment of target proteins using immobilized antibodies.

1. *Dispensing MAGAR-cN™*

A. Vortex the medium before dispensing.

B. Pipette out the required amount of medium slurry into the appropriate tube immediately after resuspension. Repeat the resuspension step between each pipetting step.

C. Dispense 50-100 µL of the homogenous medium slurry into an Eppendorf tube.

D. Place the Eppendorf tube in the magnetic rack.

E. Remove the storage solution.

2. *Equilibrating*

A. Add 300 µL **binding buffer** and resuspend the medium.

B. Remove the liquid.

3. *Binding of biotinylated antibody*

A. Add 300 µl of biotinylated antibody solution (**0.1-0.4 mg/ml; the total quantity can affect the efficiency of the immunoprecipitation**). If your solution is less than 300 µL, dilute to 300 µL with the binding buffer.

B. Resuspend the medium and incubate for 45 minutes with a thermomixer at 1000 rpm, at RT. Remove the liquid.

4. *Washing (perform this step two times totally)*

A. Add 500 µL **Washing Buffer 1** and resuspend the medium

B. Incubate for 30 second.

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C. Remove the liquid. Repeat step A and B for two times in total. This washing step can be repeated more time is the structure of the epitope to be capture depends heavily on divalent ions that can be chelated by EDTA

5. Binding of the target protein

A. Resuspend MAGAR cN™ in 50 µL **Incubation Buffer.**

B. Add 300 µL of sample. **If the sample volume is less than 300 µL, dilute to 300 µL with the Incubation Buffer**.

C. Resuspend the medium and incubate for 60 minutes with slow end-over-end mixing or by using a benchtop shaker. The incubation can be performed at room temperature or at +4°C o.n. The use of a 0.5 mL tube will reduce the available area for the undesired binding of the beads to the plastic.

D. Remove the liquid.

6. Washing

A. For a better separation place the magnetic rack with samples on ice for 5 min

B. Perform one of the two washing steps:

OPTION A

MILD WASHING (usually suggested if you do not have a strong background): Add 500 µL **Washing Buffer** 1 and resuspend the medium; remove the liquid using the magnetic rack. **Perform this step two times totally**

OPTION B

MIDIUM WASHING: Add 500 µL **Washing Buffer** 2 and resuspend the medium; remove the liquid using the magnetic rack. **Perform this step two times totally**

OPTION C

STRINGENT WASHING: Add 500 µL **Washing Buffer 3** and resuspend the medium; remove the liquid using the magnetic rack. **Perform this step two times totally**

C. Remove the liquid.

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7. Eluting

OPTION A

The streptavidin-biotin bond can be broken efficiently only by harsh denaturing conditions. Hence, dissociation of biotin streptavidin will denature both the biotinylated protein and streptavidin.

The sample can be heated at 100°C for 10 min in the presence of 1% SDS or in 1x Laemmli Sample Buffer before loading in on a SDS-PAGE gel.

OPTION B

A. Add 50 µL of Elution Buffer (This elution buffer will no break the biotin-neutravidin bond)
B. Resuspend the medium and incubate for five minutes at 80 °C
C. Place the Eppendorf tube in the magnetic rack.
D. Remove and collect the elution fraction.
E. The collected elution fraction contains the main part of the protein. If needed, repeat the elution.

Optimal parameters for protein enrichment depend on the specific biomolecules used. Hence, optimization may be required to obtain the best results.

Non-specific binding can be minimized by decreasing the amount of MAGAR cN™ *beads, increasing the amount of biotinylated protein or by optimizing the wash conditions. If proteins are bound non-specifically to the Eppendorf tube, the bead solution can be transferred to a fresh tube during the last wash step.*

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For more information, visit

www.immaginabiotech.com

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