IMMAGINA BioTECHNOLOGY s.r.l
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Via del Castel, 21 - Mattarello
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Instruction 016-01-i-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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Recommended buffers

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

Binding buffer 1	2M NaCl, 1mM EDTA, 10 mM Tris-HCl pH 7.5
Buffer buffer 2*	50 mM Tris HCl, 150 mM NaCl, 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
Elution buffer	100°C for 10 min in the presence of 1% SDS or 1x Laemmli Sample Buffer can be used) **OR** 8 M guanidine•HCl, pH 1.5

(*) see step 2c

(**) see step 4

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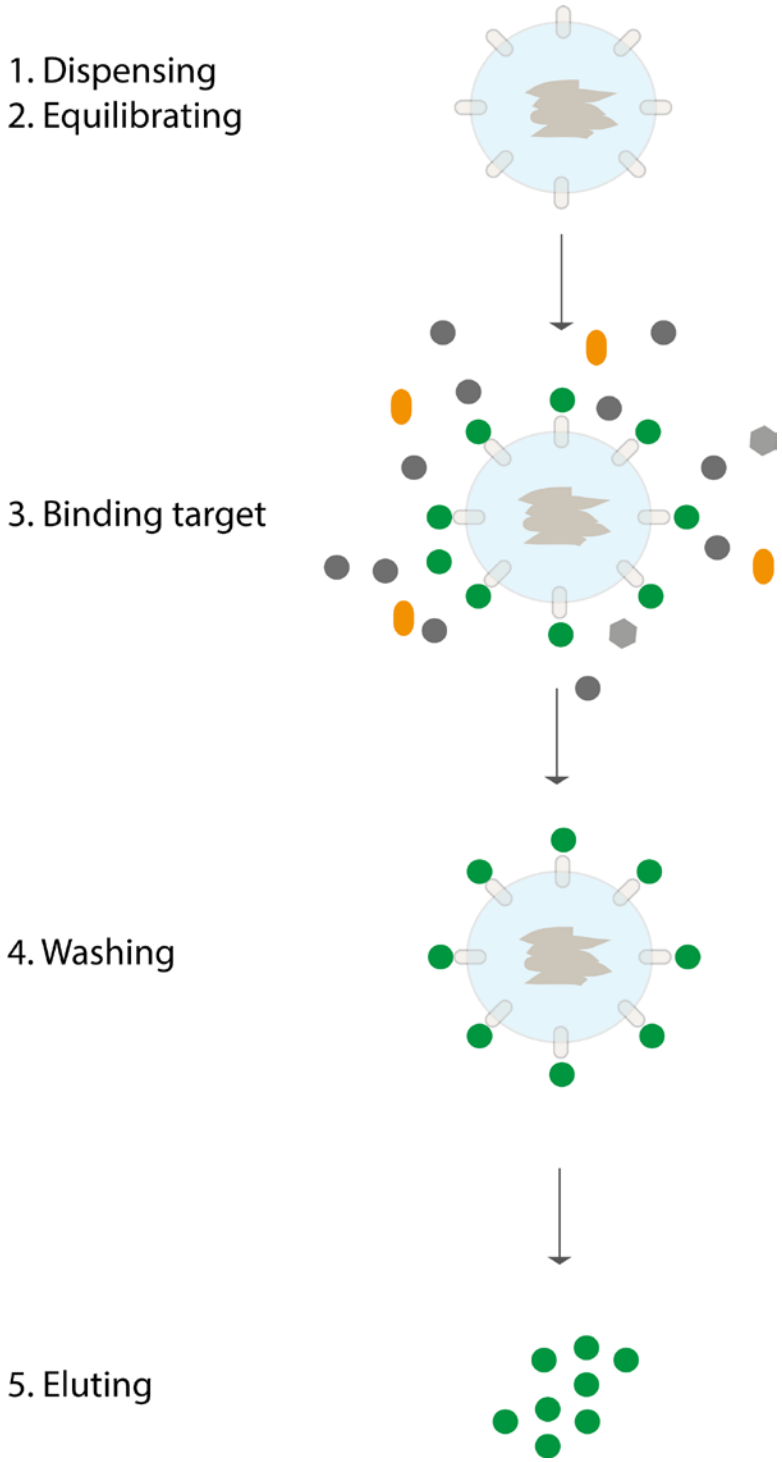

Scheme I

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Protocol for capturing and eluting biotinylated proteins (Scheme I)

This protocol is also suitable for depletion of a biotinylated protein from a sample

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 500 µL **binding buffer** 1 and resuspend the medium.

B. Remove the liquid.

C. The **binding buffer 1** can be substituted with **binding buffer 2** if the structure of the protein to be capture depends heavily on divalent ions that can be chelated by EDTA.

D. Remove the liquid.

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3. *Binding of biotinylated protein*

A. Add 300 µL of sample. If the sample volume is less than 300 µL, dilute to 300 µL with the binding buffer used in step 2c. If less volume is required, make sure that the beads stay wet all time.

B. Incubate for 60 minutes with slow end-over-end mixing or by using a benchtop shaker at > 1000 rpm. 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.

C. Remove the liquid.

4. *Washing*

OPTIONAL: For a better separation place the magnetic rack with samples on ice for 5 min

A. 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**

B. Remove the liquid.

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5. 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.**
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

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