# MagIC Beads

## RNA affinity purification protocol

This protocol describes how to perform RNA affinity purification with Magnetic Instant Capture (MagIC) Beads for the identification of proteins interacting with the target RNA.

***Product description:***
The MagIC Beads RNA affinity purification kit contains:

**Beads:**
MagIC Beads supplied as a 5 mg/ml suspension in phosphate buffered saline (pH 7,4), containing 0.05% Tween-20 and 0.02% NaN3.
The provided beads carry a pool of DNA hybridization probes covalently attached to the surface of the beads through their 5' ends. The probes are designed to hybridize specifically to the following sequence of *xOrganismx*:
xtarget RNAx:
x sequence x

The established amount of beads for a single reaction capturing a single RNA target is typically equivalent to 40pmol of capture probes. That amount of beads is optimal for the exhaustive capture of a highly expressed target RNA (e.g. GAPDH in HEK293 cells) from 40mln lysed cells. For RNA targets with lower expression levels the amount of cells used per reaction may be increased. The recommended number of cells to be used per reaction ranges from 40 to 100mln cells.

Beads information summary:

| | Number of reactions | Bead stock concentration | Probes/mg of beads | Recommended amount of beads per 40-100 mln cells |
|---|---|---|---|---|
| MagIC Beads xtarget namex | x | 5mg/ml | x pmol | xmg carrying 40pmol of capture probes (xµl of stock bead suspension) |

**Buffers:**
Three buffers are supplied with the kit: Lysis Buffer, Lysate Dilution Buffer and Wash Buffer.
All the buffers are to be kept at 4°C for storage. The components of the buffers, however, precipitate in low temperatures and they need to be re-dissolved prior to each use. It is recommended to make aliquots of the buffers after the first use to avoid the need to re-dissolve high buffer volumes each time an experiment is to be performed.

Lysis Buffer – optimized for cell lysis and solubilization of cross-linked RNA-protein complexes.
Lysate Dilution Buffer – provides conditions, which deactivate RNases and optimized probe hybridization conditions to the cellular lysate.
Wash Buffer – optimized for washing out the molecules unspecifically attached to the beads after the hybridization, while maintaining the deactivation of RNases.

***Other required materials (not provided):***
- Magnetic rack.
- Temperature controlled mixer
- 5M Dithiothreitol (DTT)
- Protease inhibitors of choice
- Buffer for elution of captured RNA-protein complexes: nuclease free 10mM Tris-HCl pH 7,5.

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***Protocol:***

Before you start

Buffer preparation:

**Lysis Buffer** – Remove the bottle from 4°C and heat it in a water bath at 45-50°C with occasional gentle shaking until all precipitated buffer components dissolve and the buffer is clear. Transfer to a fresh tube 4ml of the buffer per ml of cell pellet intended for lysis. Add 6,6µl of 5 molar DTT per ml of buffer and protease inhibitors of choice to a final concentration of 1,33x (up to 20µl per ml of buffer).
Chill the buffer on ice for 5 min prior to use. **Keep the buffer at room temperature until then, as prolonged incubations in low temperature will cause the buffer components to precipitate.** It is also advised to make aliquots of the buffer at this stage for future experiments to avoid the necessity to heat the large buffer volume every time in following experiments.

**Lysate Dilution Buffer** – Remove the bottle from 4°C and heat in a water bath at 45-50°C with occasional gentle shaking until all precipitated buffer components dissolve and the buffer is clear. Keep in room temperature until use.

**Wash Buffer** - Remove the bottle from 4°C and heat in a water bath at 45-50°C with occasional gentle shaking until all precipitated buffer components dissolve and the buffer is clear. Transfer to a fresh tube xml of the buffer per xmg of beads (xµl stock bead suspension) and add 5µl of 5 molar DTT per ml of the buffer and protease inhibitors of choice to a final concentration of 1x (up to 20µl per ml of buffer). Buffer can be stored in room temperature until needed, but prior to use for washes it should be pre-heated to 46°C.

**Elution Buffer** – prepare nuclease free 10mM Tris pH 7,5 for the elution of captured RNA-protein complexes from the beads.

Thermo blocks:

Prepare thermo blocks, which will be used at 60°C to 46°C for hybridization, 46°C for the washes and 85°C for the elution of captured molecules.

***Preparation of magnetic beads for the enrichment***

1. Place the container with magnetic beads on the bench and allow the content to equilibrate to room temperature.
2. Resuspend the particles thoroughly.
3. Transfer desired amount of beads to a fresh tube.
4. Concentrate particles on a magnetic rack and remove the liquid.
5. To wash beads resuspend them in 1ml of previously prepared Wash Buffer per xµl of stock bead suspension used and place them on the magnetic rack. Keep the beads in the buffer until the cellular lysates are prepared for hybridization.

***Preparation of the lysates for the enrichment***

1. Take desired amount of cells (recommended 40-100mln cells per xmg beads contained in xµl stock bead suspension) collected freshly or previously flash frozen in liquid nitrogen and stored in -80°C. If using frozen cells, place tubes on ice and allow them to thaw.
2. After the cells have thawed lyse them with 4 cell pellet volumes of cold Lysis Buffer containing DTT and protease inhibitors by pipetting the cells with the buffer up and down until the pellet is evenly dissolved in the buffer and incubate on ice for 10 min. **Remember: chill the Lysis Buffer on ice 5 min prior to using it for lysis, before that keep the buffer at room temperature to avoid precipitation of buffer components.**
3. Centrifuge the lysates at 4°C at 13000g for 10 min to pellet the insoluble cellular components.
4. Transfer the supernatant to a fresh tube and store it on ice.

5. Add to the lysate 313µl of room temperature Lysate Dilution Buffer per each ml of Lysis Buffer used and mix by pipetting. **From this point on the lysate can be stored at room temperature, it should not be kept in lower temperature as this may cause buffer components to precipitate.**
6. At this point it is recommended to take aside 1% of the lysate volume as an input control for later analysis.

***Enrichment of the target RNA-protein complexes***

1. Remove the Wash Buffer from the washed beads concentrated on a magnetic rack.
2. Add the processed lysate to the washed beads.
3. Place the tubes into a thermoblock preheated to **60°C** and shake at 750-1200RPM until the particles spread evenly across the lysate (confirmed visually). **The optimal RPM value will vary depending on the volume of the sample and of beads used. It is critical to provide shaking conditions, which prevent the particles from accumulating at the bottom of the tube. The optimal RPM value may be determined at this stage.**
4. Set the thermo block to shaking at 750-1200RPM with **10sec on/30 sec** off cycles and lower the temperature to **46°C**.
5. From the moment when the thermo block has actively cooled to **46°C** incubate the sample for **30 min** with **10sec on/30 sec** off cycles shaking cycles.
6. After the incubation place the tube on the magnetic rack and let the beads to concentrate for 50-60s.
7. Transfer the liquid from the beads to a fresh tube and keep it in room temperature until the next round of enrichment is to be started.
8. Add xµl of pre-heated Wash Buffer containing DTT and protease inhibitors per xmg of beads (xµl of stock bead suspension) to the beads and wash by incubating for 10 min **at 46°C** with interval mixing with **30sec on/30sec** off cycles at 750-1200RPM.
9. After the wash, concentrate the beads on a magnet for **50-60s** and discard the buffer.
10. Steps 8-9 should be repeated for total of 4 washes.
11. Resuspend the beads in a desired volume of Elution Buffer (Tris-HCl, pH 7.5). The recommended volume of Elution Buffer to be used is equal to the original bead suspension volume taken for the enrichment.
12. Heat the sample for **2 min at 85°C** in a thermo block with shaking intervals at 750RPM.
13. Quickly concentrate the particles on a magnet and transfer the liquid containing eluted RNA-protein complexes to a fresh tube and keep it on ice.
14. Quickly add the sample previously set aside after the first round of enrichment to the beads concentrated on a magnetic rack.
15. Repeat steps 3-13 for total of 3 rounds of enrichment with the same lysate sample and the same beads. After each round combine the eluted RNA-protein complexes together in the same tube. **If using more than the recommended number of cells per reaction it might be beneficial to perform up to 6 rounds of enrichment with the same sample and the same portion of beads.**
16. The eluted sample is suitable for downstream processing with RNase treatment or proteinase digest with standard reaction considerations, followed by downstream processing and analysis methods of choice.