MagIC Beads

RNA affinity purification protocol

This protocol describes how to perform RNA affinity purification with Magnetic Instant Capture (MagIC) Beads for capture of transcripts from purified RNA samples.

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 10pmol of capture probes. That amount of beads is optimal for the exhaustive capture of a highly expressed target RNA (e.g. GAPDH from HEK293 cells) from up to 5µg of DNA free RNA sample.

Beads information summary:

	Number of reactions	Bead stock concentration	Probes/mg of beads	Recommended amount of beads per 0.1-5µg of RNA
MagIC Beads xtarget namex	x	5mg/ml	x pmol	xmg carrying 10pmol of capture probes (xµl of stock bead suspension)

Buffers:
Hybridization Buffer is supplied with the kit.
The buffer should be stored at 4°C. The components of the buffer, however, precipitate in low temperatures and it needs to be re-dissolved prior to each use. It is recommended to make aliquots of the buffer after the first use to avoid the need to re-dissolve high buffer volumes each time an experiment is to be performed.

Other required materials (not provided):
- Magnetic rack.
- Temperature controlled mixer
- Bead Wash Buffer
- Buffer for elution of captured RNA-protein complexes: nuclease free 10mM Tris-HCl pH 7,5.

Protocol:
Before you start

Buffer preparation:
Hybridization 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. **Keep the buffer at room temperature until use, as prolonged incubations in low temperatures 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.
Bead Wash Buffer – prepare nuclease free buffer containing 10mM Tris pH7,5, 50mM NaCl and 0,5%(v/v) Tween-20. This buffer is used at the last stage of enrichment to wash beads carrying captured

transcripts. The purpose of the wash is to remove leftovers of the Hybridization Buffer before RNA is eluted as carryover of the components of Hybridization Buffer can inhibit downstream enzymatic reactions.

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 and 22°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 1.5ml Eppendorf tube.
4. Concentrate particles on a magnetic rack and remove the liquid.
5. To wash beads resuspend them in 1ml of the Hybridization Buffer and place them on the magnetic rack. Keep the beads in the buffer until the RNA is prepared for hybridization.

Enrichment of the target RNA-protein complexes

1. Remove the Hybridization Buffer from the washed beads concentrated on a magnetic rack.
2. Add 40µl of Hybridization buffer to washed beads and up to 2µl of DNA free RNA sample containing up to 5µg of RNA.
3. Centrifuge the tube briefly and place in a thermoblock preheated to **60°C** and shake at 1400RPM until the particles spread evenly across the sample (confirmed visually).
4. Set the thermo block to shaking at 1400RPM 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** shaking cycles.
6. After the incubation place the tube on the magnetic rack and let the beads concentrate for 50-60s.
7. Remove the liquid from the beads.
8. Add 200µl of Hybridization Buffer to the beads and wash by incubating for 10 min **at 46°C** with interval mixing with **30sec on/30sec off** cycles at 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. Add 500µl of the Bead Wash Buffer to the beads and wash by incubating for 5 min **at 22°C** with interval mixing with **30sec on/30sec** off cycles at 1200RPM.
12. After the wash, concentrate the beads on a magnet for **50-60s** and discard the buffer.
13. 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.
14. Heat the sample for **2 min at 85°C** in a thermo block with shaking intervals at 900RPM.
15. Quickly concentrate the particles on a magnet and transfer the liquid containing eluted RNA to a fresh tube and keep it on ice.
16. The eluted sample is suitable for downstream processing and analysis with any method of choice including reverse transcription.