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.

Beads information summary:

	Number of reactions	Bead stock concentration	Probes/mg of beads	Recommended amount of beads per 80-200 mln cells
MagIC Beads …	…	5mg/ml	… pmol	… µg carrying ~200 pmol of capture probes (… µl of stock bead suspension)

Buffers:
Four buffers are supplied with the kit: MagIC Lysis Buffer, MagIC Lysate Dilution Buffer, MagIC Wash Buffer I and MagIC Wash Buffer II.
All 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.

MagIC Lysis Buffer – optimized for cell lysis and solubilization of cross-linked RNA-protein complexes, including chromatin associated RNAs.
MagIC Lysate Dilution Buffer – provides conditions, which deactivate RNases and optimized probe hybridization conditions to the cellular lysate.
MagIC Wash Buffer I and MagIC Wash Buffer II – 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.

Protocol:
Before you start

Buffer preparation:
MagIC Lysis Buffer – Remove the bottle from 4°C and incubate at room temperature with occasional gentle shaking until all precipitated buffer components dissolve and the buffer is clear (the components of the buffer may or may not precipitate at low temperatures). Transfer to a fresh tube 4ml of the buffer per ml of cell pellet intended for lysis. Add 1µl of 5 molar DTT per ml of the buffer and protease inhibitors of choice to a final concentration of 1x (up to 40µl per ml of the buffer) and mix well.

Chill the buffer on ice for 5 min just prior to use. **Keep the buffer at room temperature until then, as prolonged incubations in low temperature may cause the buffer components to precipitate.**

MagIC 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 at room temperature until use. Prior to use add 1µl of 5 molar DTT per ml of buffer and protease inhibitors of choice to a final concentration of 1x (up to 40µl per ml of buffer) and mix well.

MagIC Wash Buffer I - Remove the bottle from 4°C and heat in a water bath at 40°C with occasional gentle shaking until all precipitated buffer components dissolve and the buffer is clear. Transfer to a fresh tube volume of the buffer equivalent to 4 volumes of original bead suspension used for the experiment and add 1µl of 5 molar DTT per ml of the buffer and protease inhibitors of choice to a final concentration of 1x (up to 40µl per ml of buffer). Buffer can be stored in room temperature until needed, but it is recommended to use it pre-heated to ~45-50°C for the washes.

MagIC Wash Buffer II - Remove the bottle from 4°C and incubate at room temperature with occasional gentle shaking until all precipitated buffer components dissolve and the buffer is clear (the components of the buffer may or may not precipitate at low temperatures). Buffer can be stored in room temperature until needed, but it is recommended to use it pre-heated to ~45-50°C for the washes.

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 for hybridization, 60°C for the washes and 92°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 5-10x volume of the original bead suspension of room temperature MagIC Wash Buffer II 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 or purified nuclei free of the collection buffer (recommended ~80-200 mln cells per 200 pmol of capture probes) 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 partially.
2. After the cells have thawed lyse them with 4 cell pellet volumes of cold MagIC 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 MagIC 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. The buffer components after cell lysis do not precipitate in low temperatures.**
3. Centrifuge the lysates at 4°C at 16000g for 10 min to pellet the insoluble cellular components.
4. Transfer the supernatant to a fresh tube, while avoiding the disruption of the pellet (partial disruption and carryover of the pellet is not a concern and will not negatively influence the capture, as long as the majority of it is not transferred) and store the supernatant on ice.
5. Add to the lysate 250µl of room temperature MagIC Lysate Dilution Buffer containing DTT and protease inhibitors per each ml of cleared lysate and mix by pipetting. **From this point on the lysate can be stored at room temperature for short periods of time, 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 of the efficiency of the enrichment.

Enrichment of the target RNA-protein complexes

1. Concentrate the washed beads on a magnet and remove the MagIC Wash Buffer II from them.
2. Add the processed lysate to the washed beads, remove the tube from a magnet and resuspend the beads in the lysate well by gentle pipetting.
3. Place the tube 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 size of the tube, 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 and at the same time do not lead to splattering of the lysate on the cap of the tube. The optimal RPM value may be determined at this stage.**
4. Set the thermo block to shaking at 750-1200RPM with **3sec on/30 sec off** cycles.
5. Incubate the sample for **30 min** with **3sec 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 at least **2 min**.
7. Remove the liquid from the beads.
8. It is recommended to take aside 1% of the lysate volume after the incubation with the beads as a control for later analysis of the efficiency of the target capture.
9. Add 30 µl per pmol of capture probes used of pre-heated MagIC Wash Buffer I containing DTT and protease inhibitors to the beads, resuspend them by gentle pipetting and wash by incubating for 10 min at **60°C** with interval mixing with **3sec on/30sec** off cycles at 750-1200RPM.
10. After the wash, concentrate the beads on a magnet for minimum **2min** and discard the buffer.
11. Steps 9-10 should be repeated for a total of 4 washes.
12. Add 30 µl per pmol of capture probes used of pre-heated MagIC Wash Buffer II equal to the beads, resuspend them by gentle pipetting and wash by incubating for 10 min at **60°C** with interval mixing with **3sec on/30sec** off cycles at 750-1200RPM.
13. After the wash, concentrate the beads on a magnet for at least **1min** and discard the buffer.
14. Resuspend the beads in 30 µl of room temperature Elution Buffer (10mM Tris-HCl, pH 7,5) per pmol of capture probes by gentle pipetting and directly after concentrate them on a magnetic rack for minimum **2min**.
15. Discard the buffer.
16. Resuspend the beads in a desired volume of Elution Buffer (nuclease free 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, however, volumes as low as 1/5 of the original bead suspension volume taken for the enrichment can be used.
17. Heat the sample for **2 min at 92°C** in a thermo block with shaking intervals at 600RPM.
18. 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.
19. 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.
20. It is recommended to take 1% of the volume of the eluate for the analysis of the enrichment efficiency

Analysis of the efficiency of the enrichment

1. To the 1% of input sample and 1% of the sample after incubation with the beads taken aside previously add sample 2 volumes of MiliQ water and Proteinase K to a final concentration of 2mg/ml (**Note**: in case of no crosslink control samples Proteinase K treatment is not necessary. With those samples you can proceed directly to the Phenol/Chloroform extraction).

2. To the 1% of eluate sample add ½ of the sample volume of MagIC Wash Buffer II and Proteinase K to a final concentration of 2mg/ml (**Note**: in case of no crosslink control samples Proteinase K treatment and Phenol/Chloroform extraction are not necessary. With those samples you can proceed directly to the cDNA synthesis).
3. Incubate the samples containing the Proteinase K for 1h at 55°C.
4. Perform RNA purification using standard Phenol/chloroform extraction, following standard RNA precipitation with salt and alcohol.
5. Proceed to cDNA synthesis and analyze the efficiency of the RNA capture using RT-qPCR.

Preparation of the sample for mass-spectrometry analysis

For the analysis of captured proteins the nucleic acids present in the enriched sample have to be removed. Various methods can be employed. A following recommended example involves the use of Benzonase nonspecific RNA/DNA nuclease:

1. Add following components to the sample eluted from the beads to indicated concentrations:
    - Tris-HCl pH 8,0: 20mM (minus 10mM already present in the sample)
    - $MgCl_2$: 2mM
    - TCEP: 0,5mM
    - Benzonase 40U (40U is an optimal amount for enrichment from 80mln of input cells)
2. Incubate the sample for 2h at 37°C.
3. Precipitate the proteins overnight with 10% TCA at 4°C.
4. Pellet the proteins by centrifugation at 4°C, >20,000g for 30 min.
5. Wash with 1ml of cold acetone and centrifuge at 4°C, >20,000g for 30 min.
6. Air dry the pellets to remove acetone and store at -20°C until processing for mass spectrometry.