

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

Product description:

The MagIC Beads RNA affinity purification kit contains:

Beads:

The provided targeting beads carry a pool of DNA hybridization probes covalently attached to the surface of the beads through their 5' ends.

The recommended amount of capture probes for a single reaction is 20 pmol. The volume of the original bead suspension carrying 20 pmol of probes is variable.

Consult the MagIC Beads datasheet provided with your order or the label on the bead container to calculate the volume of the bead suspension carrying 20 pmol of capture probes.

Buffers:

All buffers should be kept at 4°C for long-term storage. The components of the buffers, however, precipitate in low temperatures and need to be re-dissolved before each use.

MagIC Hybridization Buffer FA – optimized for hybridization of target RNA with probes, and RNase inhibition.

MagIC Wash Buffer I FA – optimized for washing out the molecules non-specifically attached to the probes after the hybridization, while maintaining the deactivation of RNases.

Other required materials (not provided):

- Magnetic rack.
- Temperature controlled mixer
- Nuclease-free 10mM Tris pH 7.5

Protocol:

Before you start

Buffer preparation:

MagIC Buffer Hybridization Buffer FA – Remove the bottle from 4°C and equilibrate to room temperature. Occasionally gently shake the bottle until all precipitated buffer components dissolve and the buffer is clear. Take additional care when looking for any undissolved buffer components. Re-dissolving the buffer components may be accelerated by incubating the bottle at 30-40°C if necessary. Keep at room temperature until needed.

MagIC Wash Buffer I FA - Remove the bottle from 4°C and equilibrate to room temperature. Occasionally gently shake the bottle until all precipitated buffer components dissolve and the buffer is clear. Re-dissolving the buffer components may be accelerated by incubating the bottle at 30-40°C if necessary. Keep at room temperature until needed. Keep at room temperature until needed.

Elution Buffer – prepare nuclease-free 10mM Tris pH 7,5.

Thermo blocks:

Prepare thermo blocks, which will be used at 40°C for hybridization and washes, and 92°C for the elution of the captured molecules.



Sample requirements:**Presence of genomic DNA in the sample:**

The RNA sample used for the specific target capture does not have to be free of DNA. In some cases, however, the removal of gDNA can be beneficial to the efficiency of the enrichment. It is generally recommended to perform DNase treatment before using the RNA for the capture.

The amount of input RNA:

The amount of RNA to be used for capture can vary largely. The amount of beads recommended for a single reaction (carrying 20 pmol of capture probes) has been successfully used with amounts of input RNA ranging from 10 ng to 50 µg depending on the abundance of the target RNA.

For best performance, it might be beneficial to experimentally determine the optimal amount of beads needed for a given RNA target in a specific amount of input RNA. Using too small amounts of the beads will result in incomplete capture of the target from the sample.

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, resuspend the particles thoroughly.
2. Transfer the desired amount of beads to a fresh 1.5ml Eppendorf tube (low-binding tubes are recommended).

Keep the beads in the buffer at room temperature until the RNA is prepared for hybridization.

Preparation of magnetic beads for the enrichment

1. Place the containers with magnetic beads on the bench and allow the content to equilibrate to room temperature.
2. After the bead suspension has warmed up resuspend the particles thoroughly.
3. Transfer the desired amount of beads to a fresh 1.5ml Eppendorf tube (low-binding tubes are recommended).
4. Keep the beads in the storage buffer at room temperature, until needed.

Enrichment of the target RNA

1. For every portion of beads carrying 20 pmol of the capture probes mix 15-25 µl of the RNA sample (adjust the volume with nuclease-free water if necessary) with 5 µl of MagIC Hybridization Buffer FA per every µl of the sample and mix the samples by pipetting or vortexing (the buffer is quite viscous). Keep the samples at room temperature (**do not keep the sample at a lower temperature as it will cause the components of the buffer to precipitate**).
2. Concentrate prepared beads on a magnetic rack for at least **1 min** and remove the storage buffer completely.
3. Add the RNA sample containing MagIC Buffer (90-150 µl of the sample per 20 pmol of the capture probes) directly to the beads concentrated on the magnetic rack.
4. Centrifuge the tube briefly to collect all the liquid and beads at the bottom of the tube, resuspend the beads thoroughly by gentle pipetting (make sure that all the liquid and the beads are on the bottom of the tube), and place the tube in a thermo block preheated to **40°C**.
5. Set the thermo block to shaking at **1200-1400 RPM** at **40°C**.

The optimal RPM value will vary depending on the volume of the sample. It is critical to provide shaking conditions, which prevent the particles from accumulating at the bottom of the tube, while at the same time do not lead to the sample splattering to the cap of the tube. The RPM value may have to be further adjusted if reactions are performed at volumes different from the recommended ones or in tubes of different sizes.

6. Incubate the sample for **30 min** with **5 sec on/30 sec off** shaking cycles at **40°C**.



7. After the incubation place the tube on the magnetic rack and let the beads concentrate for at least **3 min**.
8. Remove the liquid from the beads.
9. Add 400 μ l of MagIC Wash Buffer I FA to the beads, resuspend them in the buffer by gentle pipetting, and wash by incubating for 10 min **at 40°C** with interval mixing with **5 sec on/30 sec off** cycles at **750-1200RPM**.
10. After the wash place the tube on the magnetic rack and let the beads concentrate for at least **4 min**.
11. Remove the liquid from the beads.
12. Steps 9-11 should be repeated for a total of **2 washes**.
13. Resuspend the beads in 500 μ l of room temperature Elution Buffer (10 mM Tris-HCl, pH 7,5) by gentle pipetting.
14. Concentrate the beads on the magnet for at least **3 min** and discard the buffer.
15. Resuspend the beads in the desired volume (not less than 10 μ l) of Elution Buffer (10 mM Tris-HCl, pH 7.5) by gentle pipetting. Make sure that all the beads and liquid are collected at the bottom of the tube.
16. Place the tube into a thermo block pre-heated to **92°C** and incubate the samples for **2 min** with constant shaking at **600 RPM**.
17. Quickly concentrate the particles on a magnet and transfer the liquid containing eluted RNA to a fresh tube and keep it on ice.
18. The eluted sample is suitable for direct downstream processing and analysis with methods of choice including reverse transcription and various methods of sequencing library preparation. Eluted RNA can be also stored at -80°C for long periods.

Reusability of the beads:

After each round of target capture, the beads are expected to lose a few % of their binding capacity. They are, however, reusable.

To ensure no carryover of nucleic acids and proteins between experiments the beads need to be incubated in 10x volume of the original bead suspension of 10 mM Tris-HCl pH 7.5 for 2 min at 92°C after the elution of enriched molecules and before placing the beads into the storage buffer.

For the short-term storage of used beads, a buffer with the following components should be prepared:
0.05 % Tween®20, 10 mM Tris-HCl (pH 7.5 @ 25°C), 150 mM LiCl, 0.5 mM EDTA

For long-term storage, the storage buffer should be supplemented with NaN₃ to a final concentration of 0.02%.

We do not recommend re-using the beads more than 2 times.

