MagIC Beads

RNA affinity purification protocol

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 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 capturing the RNA target 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 to calculate the volume of the bead suspension carrying 20 pmol of capture probes.

Buffers:
Hybridization Buffer is supplied with the kit.
The buffer should be stored at 4°C. The components of the buffer, however, precipitate at room temperature and below and they need to be re-dissolved before each use.
The stock concentration of the buffer is 1.5x final working concentration of the buffer for each reaction should be 1x. **The components of the buffer at 1x concentration do not precipitate at room temperature.**

The buffer is optimized to deactivate the majority of known nucleases and ensure full RNA stability in a wide range of temperatures. RNA samples in 1x MagIC Hybridization Buffer do not have to and should not be kept at temperatures lower than room temperature, with exception of storing for long periods (for longer storage samples in the buffer should be kept at -20°C).

MagIC Wash Buffers I is also supplied with the kit.
The buffers should be stored at 4°C.

The components of the MagIC Wash Buffer I precipitate readily at 4°C but do not precipitate at room temperature. Before using the buffer should be treated to re-dissolve the buffer components.

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

High-quality plasticware:

Use only high-quality plasticware for the enrichment experiments to ensure optimal performance. The use of low-quality pipette tips and tubes, with a high affinity for binding nucleic acids, is particularly not recommended and may lead to the losses of both the beads and nucleic acids.

The hallmark of inappropriate plasticware can be visually observed. The sticking of the beads to the pipette tip along the length of its surface after pipetting the original bead suspension is an indicator of low-quality plastic, not suitable for use with MagIC Beads. Similarly, the tubes, which visibly retain the beads from the original bead suspension after contacting them are not suitable.

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Protocol:

Before you start

Buffer preparation:

MagIC Hybridization Buffer 1.5x concentrated – Remove the tube from 4°C and heat it at 60°C with constant agitation until all precipitated buffer components dissolve and the buffer is clear. **Use the buffer shortly after heating, as prolonged incubations at room temperature and below will cause the buffer components to precipitate.**

Buffer is supplied at 1.5x concentration to allow mixing with a sample in a **2:1** buffer-to-sample ratio to resulting 1x buffer concentration. **At 1x concentration buffer components do not precipitate at room temperature.**

MagIC Wash Buffer I – Remove the bottle from 4°C and heat it at 20-45°C with occasional shaking until all precipitated buffer components dissolve and the buffer is clear.

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

Thermo blocks:

Prepare thermal blocks, which will be used at 60°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 should be DNA-free. Large amounts of high molecular weight DNA (genomic DNA) can interfere with the target capture efficiency due to its high electric charge. It is 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. Using too high amounts may result in a slight increase in the capture of non-target RNAs.

Sample volume consideration:

The efficiency of the target capture is highest at small volumes of the reaction. For optimal results, it is recommended to use volumes of RNA samples in MagIC buffer equal to 3.75 µl/pmol of capture probes used for the capture (75 µl for the recommended 20 pmol of the capture probes).

Increasing the sample volume in relation to the amount of the capture probes above recommended may negatively influence the capture efficiency.

Decreasing the sample volume from the recommended amounts may increase the capture efficiency for some lowly abundant RNA targets.

Scalability of the reactions:

The capture reactions can be scaled up or down in a straightforward way. To scale the reactions up or down simply use a higher/smaller amount of the beads per reaction with an appropriately increased/decreased amount of buffers.

Scaling the reactions down might be useful when the downstream application does not require high amounts of the target to be recovered, for example when the capture aims to analyze the captured transcripts with RT-qPCR or sequencing workflows, which involve the PCR amplification of the recovered nucleic acids. It might be particularly useful for establishing the optimal ratio of the input RNA to the beads for lowly abundant RNA targets.

Scaling the reactions up might be necessary for the recovery of lowly abundant transcripts at the amount sufficient for direct RNA sequencing with Oxford Nanopore, where the step of the PCR amplification cannot be used.

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.

Enrichment of the target RNA

1. For every portion of beads carrying 20 pmol of the capture probes mix 25 µl of the DNA-free RNA sample (adjust the volume with nuclease-free water if necessary) with 50 µl of MagIC Hybridization Buffer and keep it 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 1x concentrated MagIC Hybridization Buffer (75 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 **60°C.**
5. Set the thermo block to shaking at 1200-1400RPM with **3 sec on/30 sec off** cycles at **60°C**.

The optimal RPM value will vary depending on the volume of the sample and beads used. 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 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 **3 sec on/30 sec off** shaking cycles.
7. After the incubation place the tube on the magnetic rack and let the beads concentrate for at least **2min**.
8. Remove the liquid from the beads.
9. Add 500 µl of MagIC Wash Buffer I to the beads, resuspend them in the buffer 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 place the tube on the magnetic rack and let the beads concentrate for at least **2min**.
11. Remove the liquid from the beads.
12. Steps 9-11 should be repeated for a total of **3 washes**.
13. After the third wash place the tube on the magnetic rack and let the beads concentrate for at least **2min**.
14. Remove the liquid from the beads.
15. Resuspend the beads in 500 µl of room temperature Elution Buffer (10mM Tris-HCl, pH 7,5) by gentle pipetting.
16. Concentrate the beads on the magnet for at least **2 min** and discard the buffer.
17. Resuspend the beads in the desired volume (not less than 10 µl) of Elution Buffer (Tris-HCl, pH 7.5) by gentle pipetting. Make sure that all the beads are collected at the bottom of the tube.
18. Place the tube into a thermo block pre-heated to **92°C** and incubate the samples for **2 min.**
19. Quickly concentrate the particles on a magnet and transfer the liquid containing eluted RNA to a fresh tube and keep it on ice.

20. 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.