MagIC Beads

RNA affinity purification protocol

ElementZero Biolabs

This protocol describes how to perform RNA affinity purification with Magnetic Instant Capture (MagIC) Beads for capture of SARS-CoV-2 RNA 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% NaN_3.
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 SARS-CoV-2 sequence.

Beads information summary:

	Number of reactions	Bead stock concentration	Probes/mg of beads	Recommended amount of beads per reaction
MagIC Beads SARS-CoV-2	x	5mg/ml	x pmol	x µg carrying 20pmol of capture probes (x µl of stock bead suspension)

Buffers:

Hybridization Buffer is supplied with the kit (labeled as **MagIC Lysis Buffer**).
The buffer should be stored at 4°C. The components of the buffer, however, precipitate in room temperature and below and they need to be re-dissolved prior to each use. It is recommended to make aliquots of the buffer after the first use (when it is still warm) to avoid the need to re-dissolve high buffer volumes each time an experiment is to be performed.
The stock concentration of the buffer is 1,33x 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 most of known nucleases and ensure full RNA stability in a wide range of temperatures. RNA samples in 1x MagIC Lysis Buffer do not have to and should not be kept at temperatures lower than the room temperature, with exception of storing for long periods of time.

MagIC Wash Buffers I and II are 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 use the buffer should be treated similarly to the MagIC Lysis Buffer to re-dissolve the buffer components before use.
The components of the MagIC Wash Buffer II should not precipitate in low temperatures. If any precipitation is observed the components of the buffer can be efficiently re-dissolved at room temperature.

Other required materials (not provided):

- Magnetic rack.
- Temperature controlled mixer
- 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 (Hybridization Buffer) 1,33x concentrated – Remove the bottle from 4°C and heat it at 40-45°C with occasional shaking until all precipitated buffer components dissolve and the buffer is clear.

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Use the buffer shortly after bringing it back to room temperature, as prolonged incubations at room temperature and below 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.
Buffer is supplied at 1,33x concentration to allow mixing with a sample in **3:1** 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 40-45°C with occasional shaking until all precipitated buffer components dissolve and the buffer is clear.

MagIC Wash Buffer II – Equilibrate the buffer to room temperature before use. If precipitation of the buffer components is observed mix it after equilibrating to the room temperature until they re-dissolve.

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 thermo blocks, which will be used at 46°C for hybridization and wash, and at 92°C for the elution of the captured molecules.

Sample requirements:

Presence of genomic DNA in the sample:

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 a capture can vary largely. The optimal capture of the target will depend on the ratio of capture probes present on the surface of the beads to the number of target molecules present in the sample. In a standard setup the beads carrying ~15 pmol of probes are sufficient for an exhaustive capture of mRNA of highly expressed housekeeping gene (for example GAPDH in human RNA sample) from at least 5 µg of total RNA sample.
The same amount of beads would be sufficient for an exhaustive capture of a transcript with lower expression value (for example MALAT1 in HEK293 cells) from at least 25µg of total RNA sample.
For optimal performance it might be beneficial to experimentally determine the optimal amount of beads needed for a given RNA target in 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 of the capture of non-target RNAs.

Sample volume consideration:

The optimal sample volume for each capture reaction is 2x-4x of the original bead suspension used.
Increasing the reaction volume above 4x of the original bead suspension used for the capture reaction is not recommended as it may negatively influence the capture efficiency.

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 well in 15 µl of MagIC Wash Buffer II per pmol of capture probes (300 µl for the recommended amount of beads carrying 15 pmol of the probes).
   Keep the beads in the buffer at room temperature until the RNA is prepared for hybridization.

<u>Enrichment of the target RNA</u>

1. Mix the DNA free RNA sample with 3x sample volume of MagIC Lysis Buffer and keep it at room temperature (**do not keep the sample at lower temperature as it will cause the components of the buffer to precipitate**).
2. Concentrate washed beads on a magnetic rack for at least **2 min** and remove the wash buffer completely.
3. Add 3-5 µl of the RNA sample containing 1x concentrated MagIC Lysis Buffer per each pmol of capture probes taken for the reaction (50-100 µl per recommended amount of the beads carrying 20 pmol of the probes) directly to the beads concentrated on the magnetic rack.
4. Centrifuge the tube briefly to collect all the liquid at the bottom of the tube and place it in a thermoblock preheated to **46°C** and shake at 750-1400RPM until the particles spread evenly across the sample (confirmed visually). <u>**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.**</u>
5. Set the thermo block to shaking at 750-1400RPM with **3 sec on/30 sec off** cycles at **46°C**.
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 **60s**.
8. Remove the liquid from the beads.
9. Add 30 µl of <u>MagIC Wash Buffer I</u> per pmol of capture probes used (600 µl for the recommended amount of beads carrying 20 pmol of the probes) to the beads, resuspend them in the buffer by gentle pipetting and wash by incubating for 10 min **at 46°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 **90s**.
11. Remove the liquid from the beads.
12. Steps 9-11 should be repeated for the total of 3 washes.
13. Add 30 µl of <u>MagIC Wash Buffer II</u> per pmol of capture probes used (600 µl for the recommended amount of beads carrying 20 pmol of the probes) to the beads, resuspend them in the buffer by gentle pipetting and wash by incubating for 10 min **at 46°C** with interval mixing with **3sec on/30sec off** cycles at **750-1200RPM**.
14. After the wash place the tube on the magnetic rack and let the beads concentrate for at least **60s**.
15. Remove the liquid from the beads.
16. Resuspend the beads in 30 µl of room temperature Elution Buffer (10mM Tris-HCl, pH 7,5) per pmol of capture probes (600 µl for the recommended amount of beads carrying 20 pmol of the probes) by gentle pipetting.
17. Concentrate the beads on the magnet for at least **2 min** and discard the buffer.
18. Resuspend the beads in a desired volume (at least 10 µl) of Elution Buffer (Tris-HCl, pH 7.5). Make sure that all the beads are collected on the bottom of the tube.
19. Place the tube into a thermoblock pre-heated to **92°C** and incubate the samples for **2 min.**
20. Quickly concentrate the particles on a magnet and transfer the liquid containing eluted RNA to a fresh tube and keep it on ice.
21. The eluted sample is suitable for direct downstream processing and analysis with various methods including reverse transcription and various methods of sequencing library preparation. Eluted RNA can be also stored in -80°C for long periods of time.