MagIC Beads

DNA affinity purification protocol

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

Product description:
The MagIC Beads DNA 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 DNA with probes, and DNase 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 DNases.

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.

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Sample requirements:

Presence of RNA in the sample:

The DNA sample used for the specific target capture should be RNA-free. It is recommended to perform RNase treatment before using the DNA for the capture.

The amount of input DNA:

The amount of DNA to be used for capture can vary largely. The amount of beads recommended for a single reaction has been successfully used with amounts of input human genomic DNA ranging from 100 ng to 6 µg.

The size of DNA fragments in the input:

The high molecular weight DNA should be fragmented to the size of choice before starting the enrichment. The initial fragmentation will determine the size of the recovered DNA fragments. For the best performance, it is recommended to use fragments up to ~30kb. Using a DNA sample with fragments larger than ~50kb is likely to severely impair the efficiency of the enrichment.

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 DNA 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 DNA

1. Mix RNA-free DNA sample (from 15 to 25 µl volume) with 5 µl of MagIC Hybridization Buffer FA per every µl of the sample volume and mix by pipetting or vortexing (the buffer is quite viscous). Keep the sample 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 DNA sample containing MagIC Buffer (90-150 µl of the sample per 50 µl of the original bead suspension used) directly to the beads concentrated on the magnetic rack.
4. Make sure that all the beads and all liquid are uniformly present at the bottom of the tube, avoid pipetting the beads with the sample.
5. Place the tube into a thermo block preheated to **92°C** and incubate for 1 min without agitation and subsequent **10-20 sec** with constant shaking at **1200-1400 RPM** to make sure that all the beads spread evenly throughout the sample.
6. Quickly transfer the denatured sample into another thermo block pre-heated to **40°C** and incubate them without agitation for **20 min**.
7. After the incubation, place the tube on the magnetic rack and let the beads concentrate for at least **4 min**.
8. Remove the liquid from the beads.

9. Add to the beads concentrated on a magnet the amount of MagIC Wash Buffer I FA sufficient to submerge all of the beads (typically **700 µl** is optimal) without disturbing them (**dispense the buffer slowly and gently, to the wall of the tube opposing the one on which the beads are concentrated**) and incubate the sample **at 40°C** without agitation for **10 min**.
10. After the wash place the tube on the magnetic rack and let the beads concentrate for at least **2 min**.
11. Remove the liquid from the beads.
12. Steps 9-11 should be repeated for a total of 2 washes.
13. Add 1ml MagIC Elution Buffer (10 mM Tris pH 7.5) to the beads concentrated on a magnet without disturbing them (**dispense the buffer slowly and gently, to the wall of the tube opposing the one on which the beads are concentrated**).
14. Remove the sample from the magnet and incubate it **at room temperature** without agitation for **5 min**.
15. Place the tube on the magnetic rack and let the beads concentrate for at least **2 min**.
16. Remove all the liquid from the beads.
17. Add not less than 12.5 µl of Elution Buffer (Tris-HCl, pH 7.5) to the beads. Make sure that all the beads and the buffer are collected at the bottom of the tube. **Avoid pipetting the beads**.
18. Place the tube into a thermo block pre-heated to **92°C** and incubate the sample for **2 min** with constant shaking at 600 RPM.
19. Quickly concentrate the particles on a magnet.
20. Concentrate the beads on the magnet for at least **2 min** and transfer the liquid containing the eluted DNA to a fresh tube.
21. Eluted DNA can be stored at -20°C for long periods. The vast majority of the eluted DNA will be single-stranded and therefore not directly suitable for use in most sequencing library preparation workflows.