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A guide to using magnetic beads

Optimizing magnetic bead specificity and binding capacity

Magnetic beads are one of the most versatile tools available within molecular biology. They are widely used for:

- Separation of specific cell, organelle, or target types
- DNA, RNA, and protein isolation
- Genetic material purification
- Size selection

Magnetic beads are used for a variety of applications including cell analysis, immunoassays, and next-generation sequencing (NGS).

While magnetic beads provide a faster and easier solution than more complex and conventional methods, two important features that require optimization for successful experiments are:

- Bead specificity, the ability to bind target molecules with accuracy.
- Binding capacity, the amount of sample bound per bed volume of beads.

This guide discusses the key considerations when using magnetic beads to ensure you achieve accurate, reproducible results.

Fig 1. Example of magnetic bead separation.

The basics of working with magnetic beads

Not only are magnetic beads available in different shapes and sizes, they also come with different magnetic properties, chemical coatings, and functional groups. It is important to know these properties before beginning to work with the bead so that you can choose a magnetic bead that suits the purpose of your experiment. Be clear on what you wish to achieve and know the attributes of your chosen target to enhance the binding, sensitivity, and specificity of an experiment. Each experiment is different and needs to be empirically optimized.

Understanding the basics of an experiment and its reagents often helps to solve bigger problems. Here are a few things to keep in mind while working with magnetic beads.

Handling magnetic beads: The magnetic property of beads determines their functionality. Try to prevent beads from drying out throughout the experiment unless instructed otherwise, as dryness can sometimes crack the bead surface and interfere with elution.

1. If required, wash beads in mild buffers before use to remove storage buffer.
2. Equilibrate magnetic beads to the solution they will be used in, following the manufacturer's recommendations to achieve good results.
3. Beads tend to settle out of solution during storage, so resuspend them before use to ensure they are homogeneous. You can do this by gently flicking the tube, maintaining it in a rotating mixer, or briefly vortexing. This helps to maintain consistent results across each experiment.
4. Be careful not to introduce bubbles at any step during pipetting as beads and samples can be lost.
5. Do not spin down magnetic beads at more than 2000 rpm, as this can change their binding properties and make them difficult to resuspend or bind to targets.

Binding beads to targets: Whether you are purifying antibodies from a crude extract, coating beads with specific antibodies, or binding targets to the beads, it is important to keep them in a rotating mixer at a low speed, around 10 to 15 rpm. Slow mixing ensures uniform binding and therefore enhanced binding capacity. Try using tubes that allow liquid to flow freely to ensure thorough mixing and binding.

Capture: To avoid unwanted loss of target DNA, RNA or protein, or carry-over of beads when removing supernatant or eluant, it is important to allow sufficient time for the beads to be pulled out of suspension.

- Allow the tubes containing the suspension to stand on the magnet for at least a minute and make a visual inspection before removing the liquid fraction.
- Ensure that the tube remains on the magnet during the pipetting process otherwise the beads may return into solution.
- Avoid disturbing the bead pellet when removing the supernatant.
- To reduce nonspecific background signals, carefully remove all the supernatant. This may involve brief airdrying steps.

Washing: When washing magnetic beads, you can reduce nonspecific binding and carry-over by increasing either the number of washes, duration of washes, or salt or nonionic detergent (such as polysorbate 20) concentrations. Be sure to use sufficient wash buffer to cover the beads.

Elution: Elute the desired product in a sufficient volume of buffer; too great a volume might result in dilute targets while insufficient buffer volume reduces elution efficiency.

All targets are different

The important aspect of any magnetic bead-based experiment is the target: its source, sample preparation, and purpose in an experiment.

Although magnetic beads allow the freedom to work with samples of varying viscosities, if they are not lysed and homogenized appropriately, your results will be inconsistent, no matter how good the protocol is.

- In general, it is better to use stringent buffers for nuclear, organellar or whole-cell extractions, and mild buffers for cytoplasmic extractions.
- When working with plant cells, tissue, and some Gram-positive bacteria, grinding or bead beating methods should be used to physically disrupt the cells and release the target into the solution to facilitate the access by the beads for binding.
- If you are working with degradable samples such as proteins and nucleic acids, make sure you use suitable inhibitors.

Performing experimental steps on ice helps to ensure the target remains intact. This will also reduce non-specific background signals from degraded samples. If you intend to use these samples in future experiments, store them at -20°C or -80°C (for long-term) with appropriate inhibitors. Do not freeze-thaw your samples more than once.

For low abundance targets, it may be necessary to concentrate the sample prior to undertaking your beadbased experiment. Depending on the target, this can be achieved with a variety of methods including concentration columns, dialysis, and re-elution in smaller volumes. Alternatively, some bead-based protocols enable the target to be concentrated as required within the protocol itself. Check the manufacturer's recommendations for more details.

DNA and RNA extraction and purification using magnetic beads

The basic steps for nucleic acid extraction are sample lysis, adsorption, washing, and elution (Fig 2).

Fig 2. The steps involved in nucleic acid extraction.

- Guanidinium thiocyanate, often added to lysis buffer, is a strong denaturant that inhibits RNase and DNase activities.
- Dithiothreitol reduces protein activity, and glycogen helps precipitate nucleic acid.
- Nucleic acid binding to beads is enhanced under high salt concentration, increased incubation time and bead volume.

Fig 3. Magnetic beads stored on a MagRack which is used for small-scale protein purification and sample enrichment with magnetic beads.

Magnetic beads for nucleic acid extraction are available with functional coatings that work via electrostatic interactions or salt- or pH-mediated charge switchable attractions. Specific nucleic acid subsets can be isolated by attaching target oligonucleotides to the bead surface.

Following binding, beads are washed with different concentrations of an ethanol-based buffer to remove contaminants. The stringency of buffer and washes can be increased depending on the level of contamination.

The nucleic acid is extracted in a suitable elution buffer or water by incubation at a high temperature (65°C for 5 min or 80°C in certain cases [1]) for better recovery. In high-throughput automation methods, the elution buffer can be heated prior to adding to the beads to increase elution efficiency.

PCR-amplified DNA products, plasmid DNA, RNA, and viral or bacterial nucleic acids can be purified for downstream applications by bead-based methods. Purity will be affected by the conditions mentioned above. Refer to the manufacturer's recommendations for specific protocols.

Magnetic beads for next-generation sequencing (NGS) library preparation

NGS library preparation encompasses:

- DNA fragmentation
- Addition of adapter sequences
- Size selection
- Library QC

Size selection is a critical step that determines downstream success and can be achieved by magnetic bead-based selection. Typically, large fragments (> 400 bp) that interfere with clustering are removed first, followed by the removal of all fragments below 150 to 200 bp. The fragment size of interest is then bound to beads, washed, and eluted. Size selection can be influenced by DNA fragmentation; if the sonication or digestion protocol used is inappropriate, selection can be inefficient.

By using different volume ratios of bead suspension to sample, different fragment sizes can be isolated, giving users flexibility for customized protocols. This can vary among beads, so refer to the manufacturer's protocol to achieve the good results. Incubate the bead-sample mix for at least 5 min to allow effective binding.

Perform washes while the beads remain in the magnetic field. Residual alcohol from wash buffer can interfere with sequencing, so air-dry samples at the end of the wash protocol to prevent carry forward. In RNA-seq protocols, magnetic beads are also used to remove particular RNA types, like rRNA, or enrich specific types like mRNA, depending on the particular experiment.

Target enrichment and NGS or hybrid capture using streptavidin beads

Streptavidin-tagged magnetic beads are being increasingly used in exome and targeted sequencing due to their strong affinity for biotin (it is known to be the strongest non-covalent biological interaction). Sheared genomic DNA is captured with a pool of target-specific biotinylated probes against the whole exome, genome, target genomic regions, specific genes, exons, or sequence stretches. Probes are 50 to 120 bp length of DNA or RNA with complementary sequences that bind to target regions to form hybridized DNA. This hybridized DNA is captured with the streptavidin-tagged magnetic beads, purified by magnetic pull-down, the target DNA is then eluted and used for library preparation and sequencing.

Hybrid capture-based target enrichment allows comprehensive analysis of variant types (single nucleotide polymorphisms, insertion/deletions, copy number variations, and structural variations) by capturing large target regions in a single experiment. This is advantageous and provides improved resolution over conventional methods like PCR and molecular inversion probes (MIPs). This method is also used to study ancient DNA (aDNA) and infectious diseases.

- Uniformly sheared genomic DNA positively influences downstream success of sequencing. Sonication is a preferred way of attaining homogeneity.
- Fragment size: Smaller fragments bind with greater specificity to probes over large fragments. Therefore, uniform shearing and fragment size selection are important for the enhanced efficacy of an experiment.
- Uniformly synthesized probes provide improved sequencing efficiency.
- Double-stranded probes maximize capture efficiency by providing more chances to capture a fragment.
- Pre-hybridization DNA amplification is recommended for samples of low integrity like clinical samples (2) and aDNA.
- Hybridize the DNA and probe in a desalted environment to maximize specificity and efficiency.

- Probe-DNA hybridization is a critical step during which temperature and time are important factors. Double stranded DNA (dsDNA) denature to single stranded DNA (ssDNA) at high temperature and convert to dsDNA at low temperature when the DNA pairs with the biotinylated probes (refer to the manufacturers recommendations to achieve good results).
- Gently vortex the probe-DNA mix every few hours for homogeneity and efficient hybridization. Certain genomic regions can be difficult to capture because of repeat sequences.
- Always wash away unbound DNA before eluting the targets.

Unhybridized DNA can form a major contaminant in sequencing and can sometimes bind to streptavidin beads and increase false positives. Work with cold buffers, increase wash stringency, time and perform washes at room temperature to help eliminate unhybridized DNA bound to streptavidin beads.

Protein purification with magnetic beads

Magnetic beads are also a convenient tool for protein purification, and are used to purify single proteins, large protein complexes, antibodies and high-throughput purifications. To begin with, it is important to adjust the volume of beads to protein in the starting material. Insufficient affinity material will reduce binding capacity, and excess binding sites can amplify nonspecific signals when sites are saturated. If your biomolecule of interest is small, more will bind per bead than for larger biomolecules. Therefore, when the size of the antibody, tag, or antigen is large, the volume of beads used can be increased to provide a sufficient binding surface. Small bead size provides a greater surface area for binding and can also be used for large molecules to maximize binding capacity per bead.

For diluted samples like cell supernatants or low-abundance proteins, larger volumes of sample need to be applied to relatively smaller bead volumes, or the samples need to be concentrated as mentioned previously. Alternatively, bead-sample incubation times can be increased. Glycerol applied to the samples might reduce nonspecific binding.

Select high-affinity tags (commonly used ones include GST, histidine, and streptavidin) while ensuring they specifically express only in your protein of interest. Elute in a buffer having ligands with higher affinity for the beads than the specific protein, or with a buffer that has a higher pH.

Magnetic beads for immunoassays

Magnetic beads provide better efficacy for target detection, pull-down, protein-protein and protein-DNA interaction studies.

Magnetic beads are often blocked by the addition of bovine serum albumin (BSA), skimmed milk, sperm DNA, gelatin, polyethylene glycol (PEG) or sera, to remove unwanted nonspecific background, but ready-to-use streptavidin blocked beads are available that do not require these additions. If a blocker is used, equilibrate the beads with lysis buffer and a low concentration of the blocker and incorporate the same blocker into the wash buffer to prevent nonspecific binding. We also recommended that you "clean" the solution containing your target first by mixing it with the beads in the absence of the antibody to reduce nonspecific binding.

Antibody selection is a very important step in immunoassays. Optimize the antibody using standard immunoblotting to identify lack of specificity, if any. Selecting a specific antibody and a high-affinity bead can greatly enhance the sensitivity of an experiment. Optimize your antigen with different tags and antibodies and select the one that provides excellent results.

Sometimes all you need is to standardize what works in a good way for your needs. The excess antibody can remain unbound and result in background signals, while insufficient concentrations will not coat the beads uniformly and can reduce target binding capacity. Contaminations from light and heavy chains of antibodies can be eliminated using magnetic bead-antibody cross-linking and elution in a low pH and non-reducing buffer.

Fig 4. SEM image showing the cauliflower-like surface of the Sera-Mag™ SpeedBeads that dramatically increases the overall surface area available for binding.

Antigen-antibody binding is determined by the level of antigen expression. This critical step needs to be standardized for different antigens against specific antibody concentrations.

- For scarce antigens, you can increase the binding time (this can also increase nonspecific binding) and concentrate the samples.
- For abundant targets, you can increase antibody concentration or bead surface area.
- Binding the antigen to the bead-antibody complex is more specific than binding the antigen-antibody complex to the bead.
- Incorporate detergent in the binding buffer to reduce nonspecific binding.
- While binding at room temperature for 10 min is sufficient, slow binding at 4°C for an optimal time of 4 h might help in certain cases.
- Unless stated otherwise, all steps of the experiment can be performed on ice to reduce lack of specificity.
- Elute at 70°C for 10 min instead of 95°C, to prevent the antibody from being released from the beads.

For sandwich immunoassays, donor and acceptor beads need to be designed carefully so they do not bind to each other. Select beads that distinctly differ in their excitation and emission wavelengths, binding specificity for the respective antigen, and are not influenced by sample or buffer-specific interferences. High biomolecular concentrations should be avoided as this overloads the beads and reduces donor-acceptor signals.

Molecular diagnostic assays that use specific oligos bound to beads for sequence capture.

Magnetic beads bound to specific sequence probes are used to detect single nucleotide polymorphisms and genetic biomarkers underlying diseases in molecular diagnostic assays. This method is also used to examine cell-free DNA in urine, plasma, breast milk and serum, and bacterial or viral infections with complementary probes. Beads bound to desired probes can be customized from the manufacturer, or they can be prepared in the lab.

As diagnostic samples can be from various sources, the most common issues are:

- Contamination
- Variable quantity of DNA or RNA
- Degradation
- Presence of inhibitors

It is important to ensure that the sample to be detected is intact and that samples like blood, urine, and plasma, are processed immediately for better recovery of nucleic acids.

The specificity and efficiency of the beads in diagnostics are determined by the selected probe, incubation time, and sample abundance. Highly specific and nonoverlapping sequence oligo probes need to be designed for exceptional results. Nucleic acid-bead incubation times should be standardized to allow efficient binding and sensitive detection. In the case of low abundance samples, PCR amplification or sample concentration can enhance detection efficiency. Magnetic beads bound to capture probes and a surface-enhanced Raman scattering (SERS) nanoplatform is a useful method that provides faster results in molecular diagnostics.

Optimize and standardize your protocols to achieve exceptional results

Magnetic beads are versatile and often provide a faster and easier solution than more complex and conventional methods. The points discussed here should help enhance binding capacity and specific binding across different experiments when selecting and using magnetic beads. Certain protocols might require more optimization, depending on the characteristics of the components and requirements of an experiment. Optimizing and standardizing your protocols will yield the most consistent, reliable results, with exceptional efficiency.

Reference

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2. Mamanova L. *et al.* Target-enrichment strategies for next-generation sequencing. *Nat Methods*. 2010 Feb;7(2):111-8. doi: 10.1038/nmeth.1419. PMID: 20111037.

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