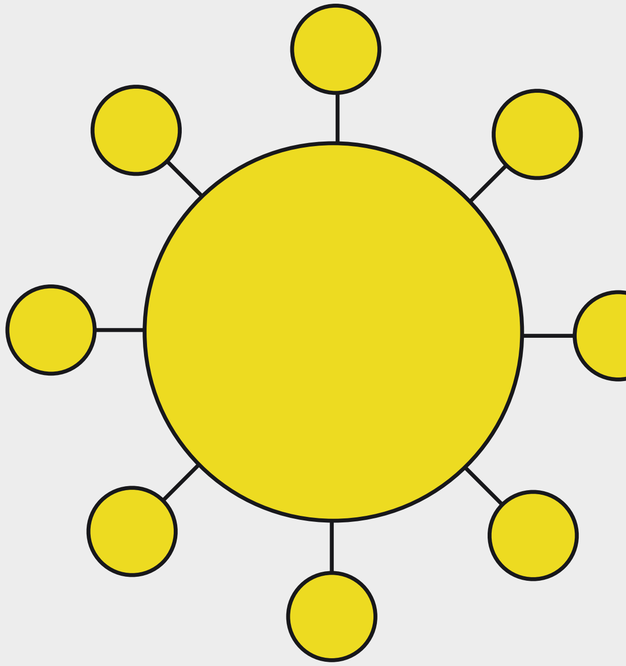
eBook

A troubleshooting guide to using magnetic beads

Optimize and standardize your protocols to achieve exceptional results

cytiva

Magnetic beads are one of the most versatile tools available within molecular biology



They are widely used for:

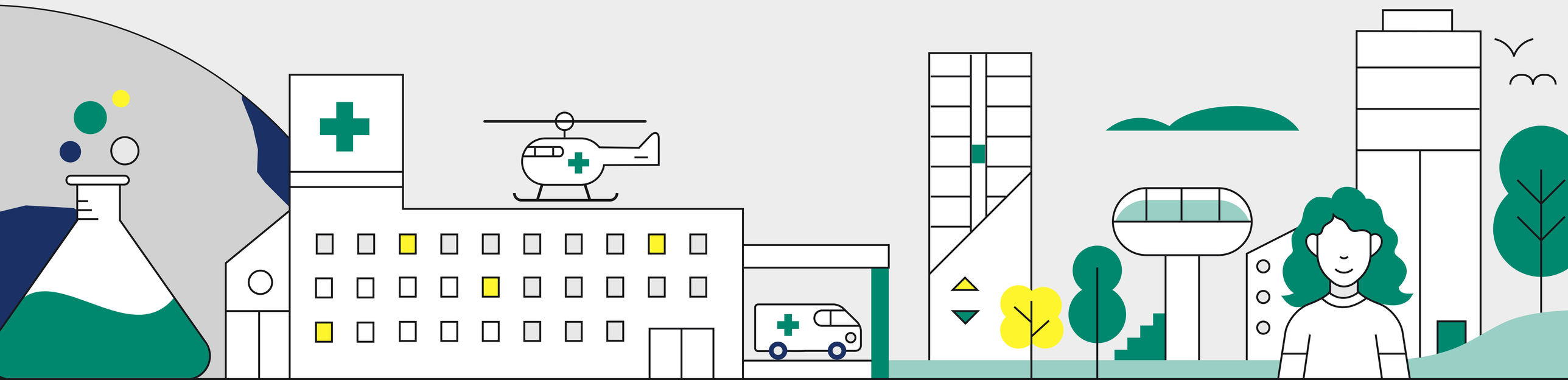
- Separation of specific cell, organelle, or target types
- DNA, RNA, and protein isolation
- Genetic material purification
- Size selection

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.



01

Working with magnetic beads

The basics of working with magnetic beads

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 air-drying 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.

Surface chemistries and applications

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.

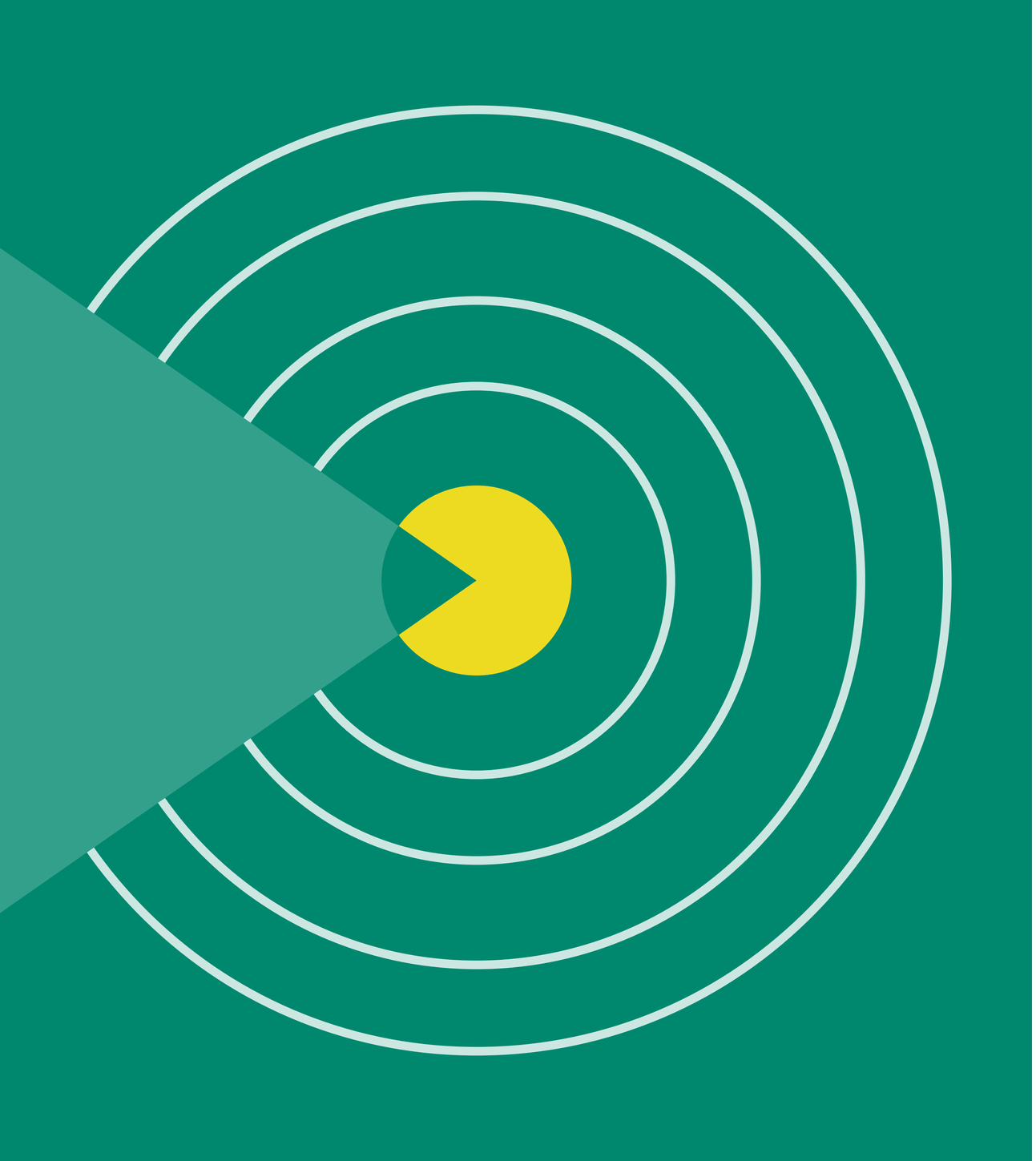
Magnetic beads come in a range of surface chemistries and sizes to meet different application requirements. Table 1 gives an overview of the various magnetic bead types with their key properties and applications.

Table 1. Comparison of magnetic bead surface chemistries and applications

Type	Properties	Applications	Variations
Carboxylate-modified magnetic beads	Can associate with nucleic acids for direct capture. Surface suitable for conjugation through covalent bonding. Can capture molecules containing amino groups.	Conjugation or direct binding applications: • Covalent attachment • Affinity purification and pull-down • Nucleic acid isolation and purification • NGS size selection	High-speed version available
Amine-blocked magnetic beads	Surface suitable for conjugation through covalent bonding. Non-surfactant, non-protein-blocked surface. Low nonspecific binding.	Conjugation applications, similar to carboxylate-modified beads.	High-speed version available
Oligo(dT)-coated magnetic beads	Hybridizes with mRNA poly-A tails. High colloidal stability.	mRNA binding applications: • mRNA extraction and purification • RT-PCR • cDNA library construction • Subtractive hybridization • NGS (RNA sequencing)	

Type	Properties	Applications	Variations
Streptavidin-coated magnetic beads	Binds biotinylated ligands such as proteins, nucleic acids, and peptides. Covalently bound streptavidin coating. Fast reaction kinetics. Low nonspecific binding. High throughput and precision.	Immunoassay and molecular biology applications: • Sample preparation and assay development for genomics and proteomics.	High-speed version available Biotin binding ranges: • 2500 to 3500 pmol/mg • 3500 to 4500 pmol/mg • 4500 to 5500 pmol/mg
Streptavidin-blocked magnetic beads	Binds biotinylated ligands such as proteins, nucleic acids, and peptides. Non-surfactant, non-protein-blocked surface. Lower nonspecific binding than streptavidin-coated beads via additional blocking of nonspecific binding sites.	High-specificity biotin binding applications Molecular and immunodiagnostics NGS library preparation	High-speed version available
NeutrAvidin-coated magnetic beads	Binds biotinylated ligands such as proteins, nucleic acids, and peptides. Fast reaction kinetics. Low nonspecific binding. High throughput and precision.	Alternative to streptavidin in immunoassay and molecular biology applications: • Sample preparation and assay development for genomics and proteomics.	High-speed version available Biotin binding range: • 3500 to 4500 pmol/mg
Protein A/G magnetic beads	Binds IgA and IgG proteins Coating based on IgA/IgG fusion protein. Broad binding capabilities.	Antibody isolation applications: • Affinity purification and pull-down • Immunoprecipitation	
SeraSil-Mag™ silica-coated magnetic beads	Reversibly binds nucleic acids based on salt concentration. Monodisperse particles with narrow size ranges of 400 µm or 700 µm.	Applications with low sample amounts Nucleic acid extraction for molecular diagnostics applications such as qPCR.	
His-Mag Sepharose™ Ni magnetic beads	Highly cross-linked spherical agarose (Sepharose), including magnetite IMAC immobilized with nickel	Small-scale purification and screening of histidine-tagged proteins from different sources	

Type	Properties	Applications	Variations
His Mag Sepharose excel magnetic beads	Strip-resistant ligand with strongly bound nickel for immobilized metal ion affinity chromatography (IMAC)	Small-scale capture and purification of histidine tagged proteins secreted into eukaryotic cell culture supernatants	
NHS Mag Sepharose magnetic beads	Coupling of antibodies, aptamers, and proteins through primary amino groups on the molecules to the NHS ligand on NHS Mag Sepharose	Enrichment of target protein for further downstream analyses such as mass spectrometry (MS) and electrophoresis techniques	
Protein A Mag Sepharose magnetic beads	Maximum binding capacity due to dense coating of Protein A. Optimized capacity for enrichment or immunoprecipitation requiring only low amounts of antibody needed.	Enrichment of target proteins via immunoprecipitation or pulldown assays. Optimized for downstream analyses such as mass spectrometry (MS) and electrophoresis techniques.	
Protein A Mag Sepharose Xtra magnetic beads	Maximum binding capacity due to dense coating of Protein A	High capacity small-scale purification and screening of monoclonal and polyclonal antibodies from various species	
Protein G Mag Sepharose magnetic beads	Maximum binding capacity due to dense coating of Protein G. Optimized capacity for enrichment or immunoprecipitation requiring only low amounts of antibody needed	Enrichment of target proteins via immunoprecipitation or pulldown assays. Optimized for downstream analyses such as mass spectrometry (MS) and electrophoresis techniques.	
Protein G Mag Sepharose Xtra magnetic beads	Maximum binding capacity due to dense coating of Protein G	High capacity small-scale purification/screening of monoclonal and polyclonal antibodies from various species	
Streptavidin Mag Sepharose	Utilizes strong interaction between biotin and streptavidin ligand immobilized on magnetic beads	Enrichment of target proteins through immunoprecipitation and purification of biotinylated biomolecules	



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.

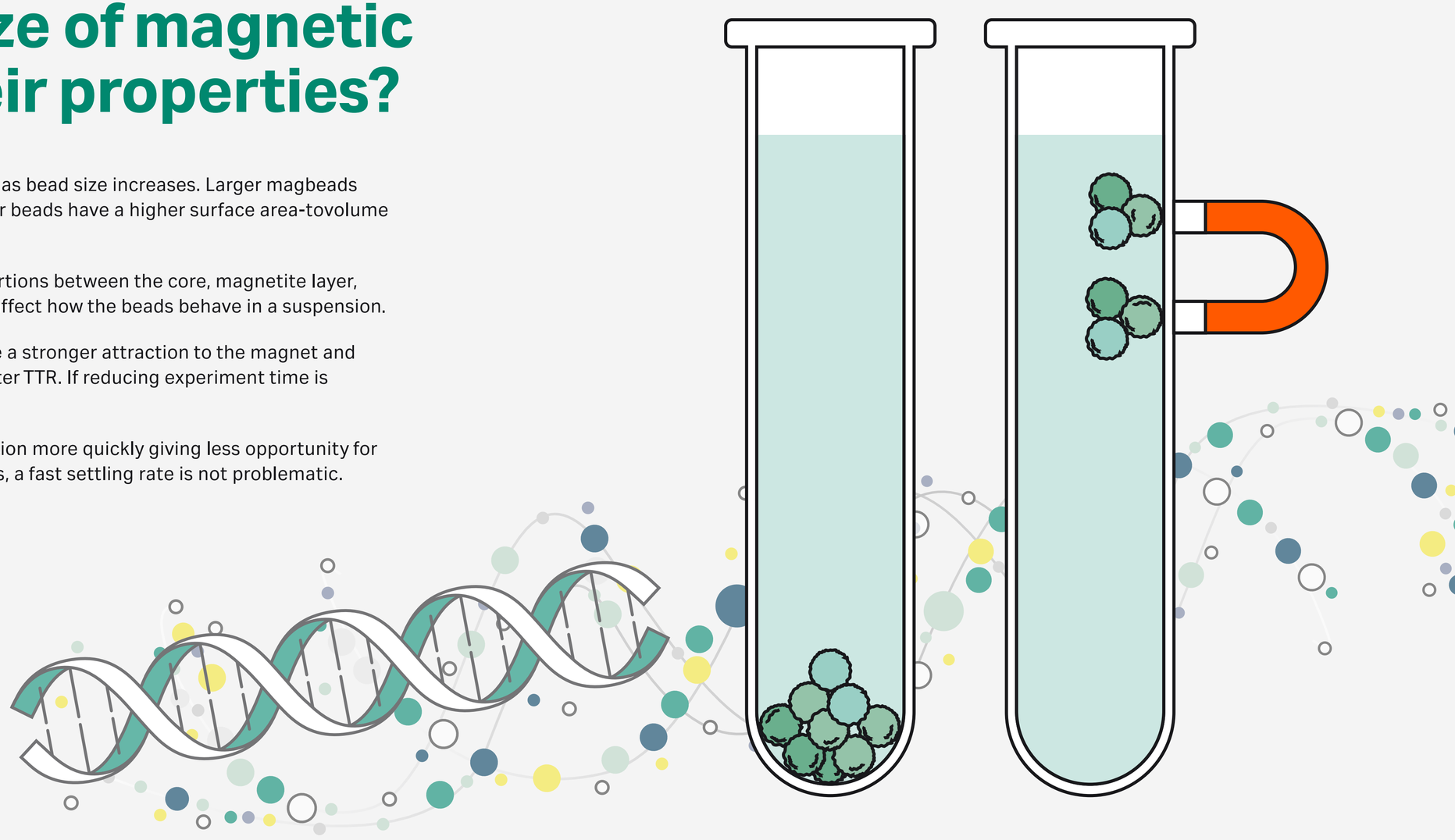
- In general, it is better to use stringent buffers for nuclear, organellar, or whole-cell extractions and use mild buffers for cytoplasmic extractions.
- When working with plant cells, tissue, and some Gram-positive bacteria, grinding or bead beating methods should be used to physically disrupt the 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 nonspecific 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 bead-based 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.

How does the size of magnetic beads affect their properties?

- **Physical properties:** The surface area increases as bead size increases. Larger magbeads have a lower surface-to-volume ratios and smaller beads have a higher surface area-tovolume ratio.
- **Density:** Increasing bead size changes the proportions between the core, magnetite layer, and polymer layer. The density of magbeads can affect how the beads behave in a suspension.
- **Time to result (TTR):** Larger beads typically have a stronger attraction to the magnet and travel faster through viscous liquids so have a faster TTR. If reducing experiment time is important, using larger beads could be an option.
- **Settling rate:** Larger beads settle out of suspension more quickly giving less opportunity for your target to bind to them. For some applications, a fast settling rate is not problematic.



Top tip: Match your bead size to your target size. Use larger beads for larger molecules such as proteins and cells and use smaller beads for smaller molecules such as nucleic acids.

02

Applications using magnetic beads

DNA and RNA extraction and purification using magnetic beads

The basic steps for nucleic acid extraction are sample lysis, adsorption, washing, and elution (Fig 1).

Silica resins or silica-coated magnetic beads use chaotropic salts to disrupt hydrogen bonds and bind nucleic acids, enabling contaminants to be washed away. The chaotropic agents help the DNA dehydration and bridge the bead surface and the nucleic acid with their divalent cations. This bridging allows the negative charges of both the bead surface and nucleic acid to be overcome and it is a reversible mechanism.

- 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.
- Chaotropic agents help DNA dehydration and binding to bead surface.

Fig 1. The steps involved in nucleic acid extraction.

The functional coatings on the beads work via electrostatic interactions or salt- or pH-mediated charge switchable attractions (Fig 2). 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.

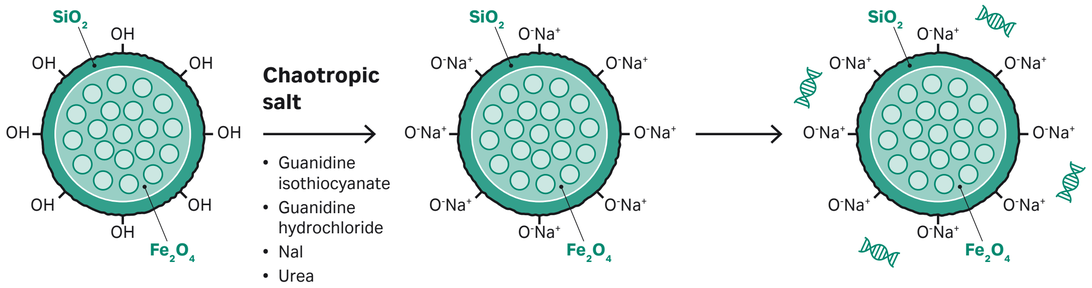


Fig 2. Chaotropic agents in nucleic acid extraction with magnetic beads

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. These ratios 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.

Library normalization with magnetic beads

The idea behind magnetic bead-based normalization is that a given volume of beads can bind a consistent quantity of nucleic acid molecules. That is, if there are enough molecules in each library to saturate the beads, an essentially equimolar quantity of library fragments will bind and be retained from each sample (Fig 3). All unbound molecules are then washed away so that each library is represented by just the bead-bound molecules. The normalized sample is then eluted from the beads for inclusion in the library.

There are several coating options available to suit any given application: carboxyl- and silica-coated magnetic beads for generic, nonspecific binding based on buffer conditions; oligo(dT)-coated beads for binding mRNA; and streptavidin-coated beads for binding biotinylated samples.

This approach is reasonably straightforward, and studies in recent years have indicated that bead-based normalization produces more consistent read depth than several existing quantitation-based methods. Illumina has exploited this approach for normalization, modifying its transposon-based "tagmentation" system for NGS library prep to use magnetic beads.

The bead-based approach, however, can be wasteful: the number of molecules in each library needs to equal or exceed the binding capacity of the beads, with the excess discarded. If your sample is precious or in short supply, it might be worth taking the extra time for qPCR-based quantitation.

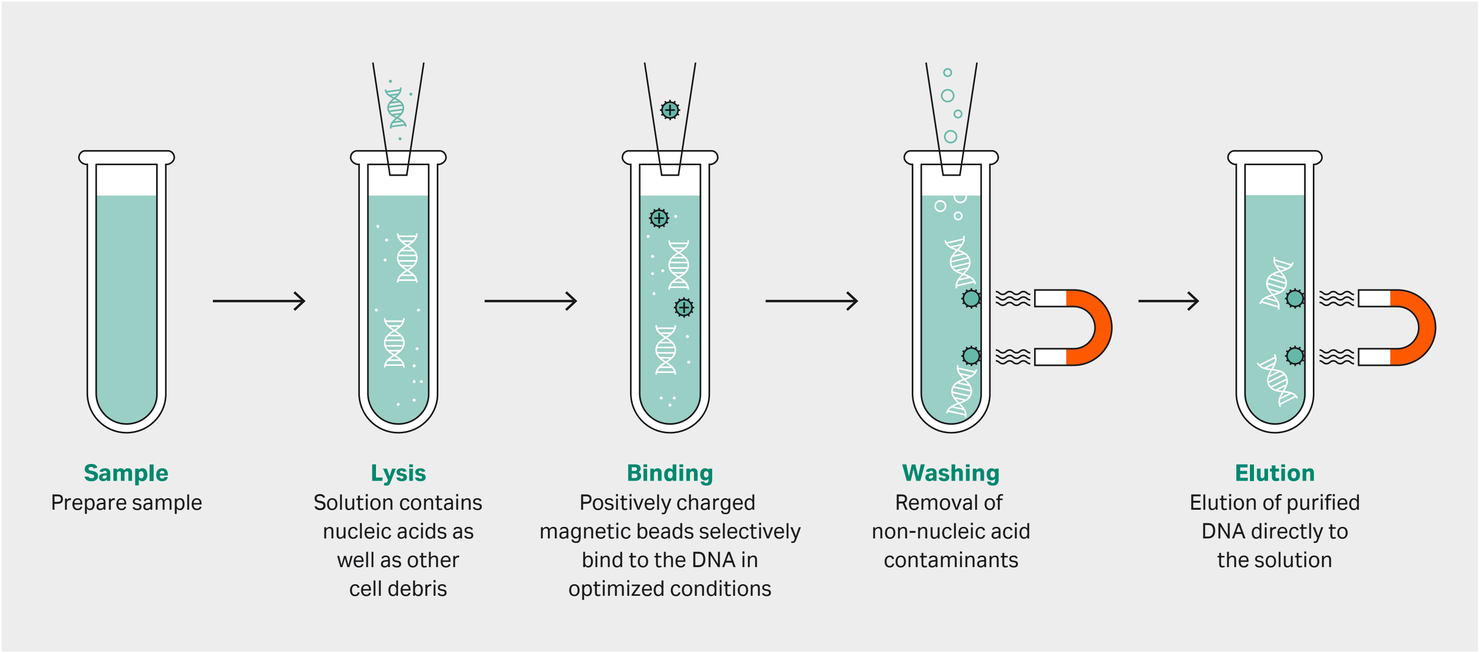


Fig 3. A given volume of beads can bind a consistent quantity of nucleic acid molecules in a four-step process from sample to elution. First, the same volume of magnetic particles is added to each sample and binds to target molecule. Then, a magnetic field is applied to capture the beads with the bound target, anad the remainder of the sample is washed away. Finally, the normalized sample is released from the magnetic particles for inclusion in the library.

For more information about the importance of DNA normalization and the range of methods, see our blog: DNA library normalization for NGS: why and how.

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 noncovalent 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 and purified by magnetic pull-down, and then the target DNA is 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.

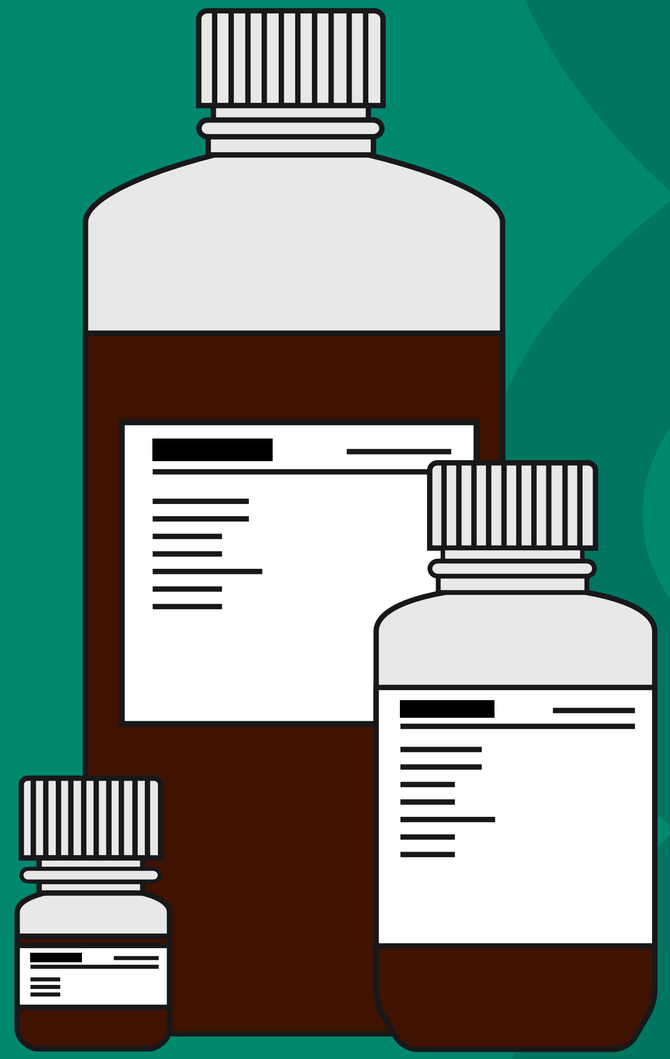
Unhybridized DNA can form a major contaminant in sequencing and can sometimes bind to streptavidin beads and increase false positives. Work with cold buffers, increase wash stringency and time, and perform washes at room temperature to help eliminate unhybridized DNA bound to streptavidin beads.

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, and antibodies for 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.

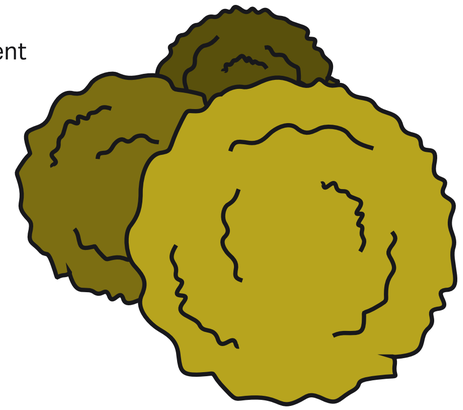


Fig 4. Illustration showing the cauliflower-like surface of the Sera-Mag™ SpeedBeads that dramatically increases the overall surface area available for binding.

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 (which 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 and 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

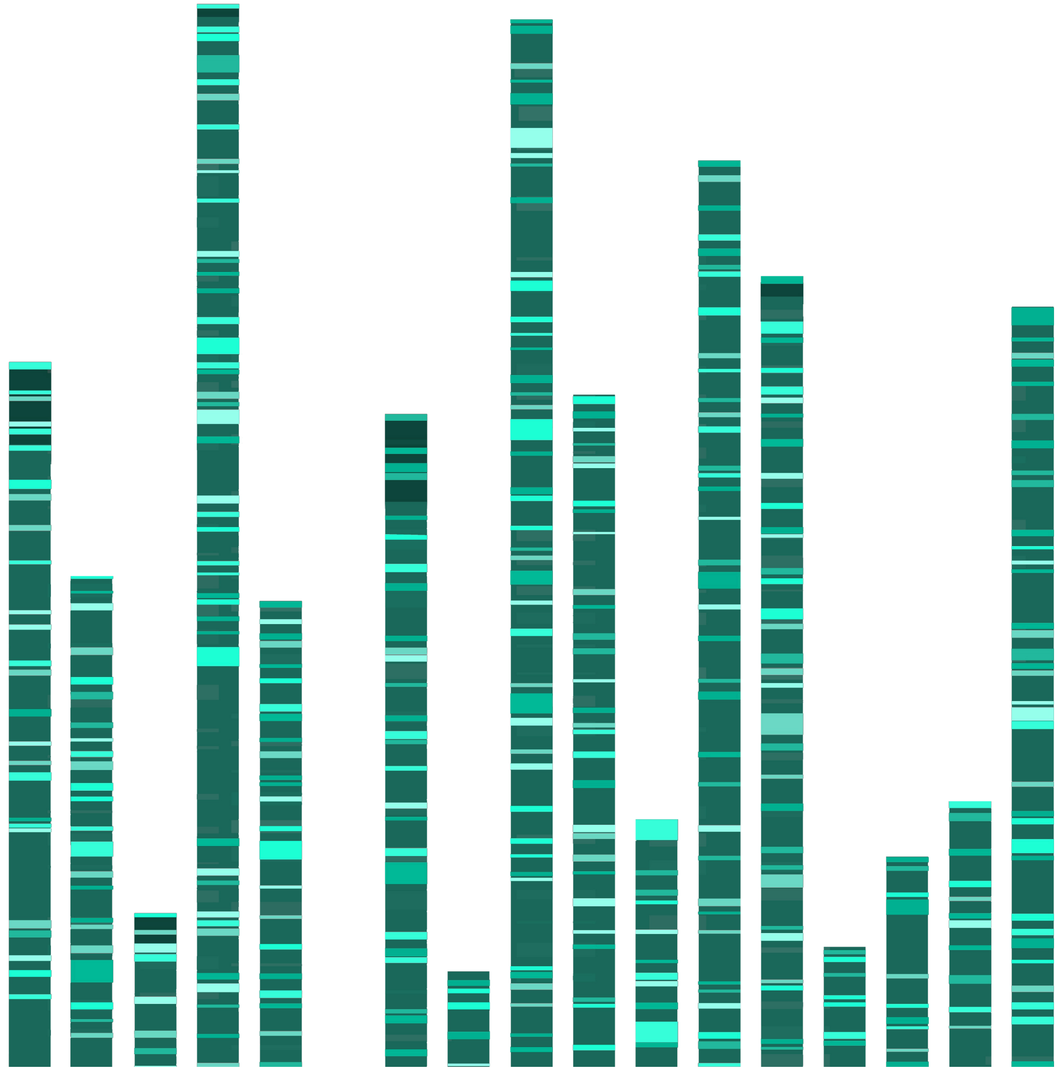
Magnetic beads bound to specific sequence probes are used to detect single nucleotide polymorphisms and genetic biomarkers underlying diseases in molecular diagnostic assays. This method is also used to examine cell-free DNA in urine, plasma, breast milk, and serum, and bacterial or viral infections 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.

A magnetic bead for every need with bead conjugation

The surface of magnetic beads can be coated in a variety of ligands to bind a specific target molecule. By exploiting this type surface modification, magnetic beads offer an exceptionally versatile tool for biomolecule isolation.

Magnetic bead conjugation: how does it work?

By altering the surface properties of magnetic beads — commonly known as surface functionalization — it is possible to be highly specific about the types of biomolecules the beads will bind. Achieved by conjugating a specific ligand suited for the binding or functionality needs, magnetic beads offer an extremely flexible platform that can be tailored for specific applications.

Magnetic beads can be conjugated with a wide variety of ligands due to the variety of different compositions, surface chemistries, and size possibilities of beads. Ligands can be bound covalently and noncovalently and link either directly to the bead surface or indirectly through a reactive intermediate. Figure 5 shows a few possible functionalization examples including common functional groups, proteins, antibodies, and nucleic acids.

Magnetic bead surfaces modified with functional groups can facilitate the attachment of ligands by both adsorption and covalent bonds. For example, proteins are rapidly adsorbed onto the surface of carboxylate-modified particles, mediated by hydrophobic and ionic interactions.

There are several possible approaches to conjugating a ligand to a magnetic bead. Typically, bead surfaces are first functionalized, which involves coating the beads to present a functional group, enabling the attachment of a specific ligand. Then, the ligand which will bind specifically to the molecule of interest can be conjugated to the functional coating.

Two common functional surface chemistries used in conjugation applications include carboxylate-modified and amine-blocked magnetic beads.

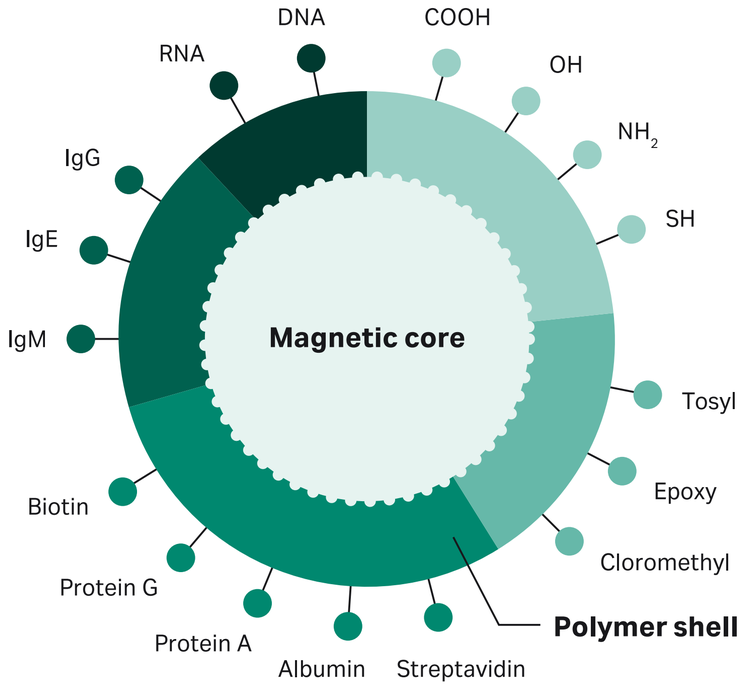


Fig 5. Magnetic bead surface functionalization examples.

Modified surfaces

Magnetic bead surfaces modified with functional groups can facilitate the attachment of ligands by both adsorption and covalent bonds. For example, proteins are rapidly adsorbed onto the surface of carboxylate-modified particles, mediated by hydrophobic and ionic interactions.

While this method is simple, the relatively weak interactions can result in challenges of instability. Additionally, the final orientation of the ligand when adsorbed onto the surface cannot be specified, potentially leading to an increase in nonspecific binding.

To overcome stability and specificity problems, proteins can also be covalently attached to the surface of, for example, carboxylate-modified magnetic beads. Taking this approach requires first activating the carboxyl groups with water soluble carbodiimide 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC); which reacts with free amino groups of the adsorbed protein to form amide bonds. This chemistry can be utilized in either a quick one-step protocol or as a two-step process for better control.

Pre-activated surfaces

Pre-activated surfaces, such as tosyl, epoxy, and chloromethyl groups, simplify things further by allowing for very stable covalent coupling without the need for an activation step. In turn, attachment protocols are typically straightforward, requiring a single incubation step in the correct buffer at the appropriate temperature and pH.

Both the chemical group and the incubation conditions will dictate what can be bound. For example, at neutral pH, tosyl groups will bind to sulfhydryl groups, whereas a more basic pH is needed for binding amino groups. Similarly, epoxy groups will bind thiol groups at a slightly basic pH and amino groups under higher pH conditions.

This pH-dependent binding provides a useful level of flexibility binding ligands to pre-activated surfaces with the caveat that binding might have less specificity compared to the modified surfaces.

Expanding applications with conjugated magnetic beads

Magnetic beads can now be conjugated with an exceptional variety of ligands ranging from antibodies, antigens, peptides, and proteins to enzymes, nucleic acids, and carbohydrates. Consequently, it is possible to target an extraordinary diversity of biomolecules and, therefore, applications.

Indeed, conjugated magnetic bead systems have been utilized for everything from benchtop research, drug screening, and diagnostics to food production and wastewater treatment. Additionally, the ability to couple magnetic beads with various biosensing devices and microfluidic systems adds further possibilities to the miniaturization of detection and separation technology.

Diagnostics: protein and magnetic bead conjugated antibody

Potentially one of the most exciting applications for conjugated magnetic beads is diagnostics. Broadly, by concentrating, isolating, and/or purifying various target analytes, high quality samples containing concentrated target molecules can be made readily available for diagnostic analysis.

For example, the concentration and purification of pathogenic microorganisms by antibody-conjugated magnetic bead separation circumvents the need for laborious and time-consuming cell enrichment processes like bacterial cell culture.

Magnetic beads can also be used more directly in diagnostics. For instance, magnetic immunoassay (MIA) is a type of diagnostic immunoassay that exploits magnetic beads for the detection of a specific analyte.

In MIA, either an antigen or antibody is conjugated to a magnetic bead. If an antibody is paired with the magnetic bead, its corresponding antigen (e.g., virus, toxin, bacteria) will bind when the magnetic bead is placed in the sample. A magnetic reader can then measure the magnetic field change induced by the beads and, because the signal is proportional to the analyte concentration in the sample, be used to give a quantitative diagnosis (3).

This system offers several advantages over traditional methodologies such as ELISA or Western blot. Primarily, MIA is conducted in a liquid medium whereas ELISA and Western blots require a stationary medium to bind the desired target. A liquid medium-based detection system allows measurement within the model system and, in turn, can provide a more accurate result.

As a final example, immunoprecipitation (IP) of proteins from cell lysates is a common method used to enrich and purify proteins for research and diagnostics. Protein A, Protein G, and Protein A/G are commonly used in IP experiments as they specifically recognize IgG antibodies, an important and common serum antibody in animals.

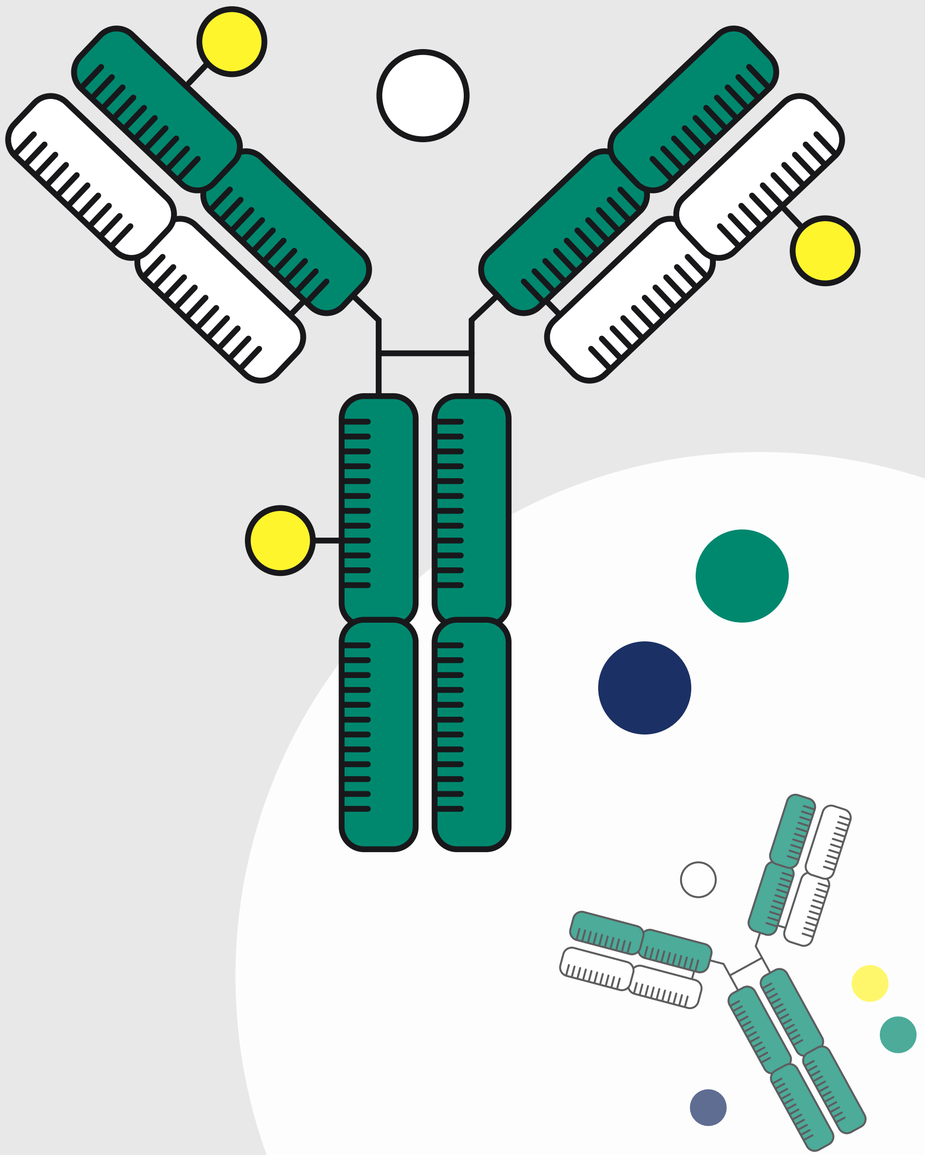
Traditional IP protocols targeting IgG employ a combination of agarose beads conjugated to either Protein A, G, or A/G and centrifugation. However, agarose and Sepharose beads with a magnetic core — such as our Protein A Mag Sepharose Xtra beads — offer a simple and efficient alternative that generally gives lower background binding than agarose beads.

As an example of this, Burbelo *et al.* (2017) demonstrated how magnetic beads modified with protein A/G could bind antibody-luciferase-labeled antigen complexes to simply and rapidly measure specific antibody levels in a variety of infectious and autoimmune disorders (4). In the case of Sjögren's syndrome, saliva samples were taken and only required approximately one minute per assay, showing the potential of this type of test at point-of-care.

Drug discovery and screening: conjugated enzyme magnetic beads

Enzymes are a common drug target because they are highly susceptible to inhibition by low molecular weight, small, drug-like molecules. However, identifying potential drug targets for a given enzyme requires lengthy, complex, and expensive pharmacological assays.

Additionally, while the majority of approved drugs are derived from natural origins, therapeutically active components in natural products are often found at extremely low concentrations. Attempting to isolate or even just detect these potentially useful compounds is difficult, if not impossible, with traditional separation and analytical methods.



Affinity-based screening approaches circumvent the need to separate potentially important compounds from a complex mixture and can be instead analyzed as a whole. Enzyme-conjugated magnetic beads are beginning to show great promise in affinity-based screening, allowing quick separation and identification of active components from complex mixtures. Additionally, they can be integrated with mass spectrometry and liquid chromatography to help clarify the structures of these active components.

For example, neuraminidase — an enzyme proposed as a potential therapeutic target for influenza — was immobilized onto the surface of magnetic beads to screen for potential inhibitors (5). Based on enzymatic activity, the researchers were able to identify four compounds as inhibitory ligands of neuraminidase from a botanical matrix.

In other applications, enzyme immobilization onto magnetic beads offers a convenient way to control and reuse enzymes. In addition, magnetic beads can improve the performance of enzyme analytical assays as they provide a relatively large number of binding sites, resulting in improved enzyme kinetics (6).

Detection of specific sequences: oligonucleotide conjugated magnetic beads

Quick, simple, and efficient methods for the detection of DNA and RNA in clinical, environmental, and research settings are extremely desirable. Not only can detection of specific fragments of DNA or RNA be used to diagnose disease, but it can be employed to identify contamination of food and water or help answer research questions.

Magnetic beads have become an important part of many next generation sequencing (NGS) workflows, where total DNA and RNA is captured, or DNA or RNA is selected based on size. However, it is also possible to conjugate specific oligonucleotide probes to magnetic beads to facilitate the detection of target DNA or RNA.

The sandwich hybridization DNA or RNA capture technique can be coupled with magnetic beads to generate a rapid DNA and RNA detection method. First, oligonucleotide probes that are complementary to the target DNA or RNA are designed and synthesized. One probe is conjugated to a magnetic bead and the other may be conjugated to a signal molecule, such as a fluorescent tag. When the conjugated probes are mixed with a sample, any target DNA or RNA will hybridize. Subsequently bound target DNA/RNA can be isolated using magnetic separation and the fluorescent signal measured to confirm its presence.

Catalog or custom?

Guided by customer requirements, our custom services team develops and manufactures magnetic beads conjugated to meet customer specifications, coupling a specific peptide protein, antibody, or oligonucleotide as well as a fluorescent dye to the surface of high-quality Sera-Mag beads.

References

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03

Procedures and protocols

Protocol: Reproducible protein and peptide cleanup for mass spectrometry with Sera-Mag Carboxylate SpeedBeads by SP3

Joseph D. Card, Guillaume Adelmant, and Jarrod A. Marto

This protocol has been developed by Joseph D. Card, Guillaume Adelmant, and Jarrod A. Marto of the Dana-Farber Cancer Institute Inc., Brigham and Women's Hospital, and Harvard Medical School, MA, USA. This note is not and should not be construed as an endorsement of any product.

Protein and peptide single-pot solid-phase-enhanced sample preparation (SP3) with Sera-Mag speedbead carboxylate-modified magnetic particles is a novel technique that provides significant cost and time savings over traditional sample cleanup methods such as batch-mode chromatography, solid-phase extraction, and spin-filters enabling the fast and efficient removal of a wide range of detergents, salts, and other contaminants from protein and peptide samples prior to mass spectrometry analysis.

https://cdn.cytivalifesciences.com/api/public/content/digi-34053-pdf

Protocol: Simple immunoprecipitation using paramagnetic beads

A major challenge in analytical-scale protein purification is to selectively capture and isolate low abundant proteins from a complex sample with several thousands of other proteins. Immunoaffinity purification, or immunoprecipitation, is an ideal technique for capture and concentration of a broad range of proteins using specific antibodies. Magnetic beads (magnetite emulsified agarose) have been tested in three different applications. These beads combine the large binding capacity of Sepharose agarose with magnetic properties to make it possible to work in low microliter volumes with negligible loss of the beads.

https://cdn.cytivalifesciences.com/api/public/content/digi-14394-pdf

Protocol: Simple immunoprecipitation of phosphorylated proteins from pervanadate stimulated CHO cells using paramagnetic beads

By using a small amount of magnetic beads with highly selective antibodies, both enrichment and concentration of phosphorylated proteins is achieved resulting in an increased sensitivity and efficiency during microscopy (MS) analysis. This paper shows how Mag Sepharose technology allows the capture of low abundant proteins from large amounts of starting samples and elution in MS-suitable volumes.

https://cdn.cytivalifesciences.com/api/public/content/digi-15584-pdf

Procedure: Coupling procedure for Sera-Mag blocked amine magnetic particles

This procedure should be optimized to produce best results as coupling efficiency and particle performance is protein specific.

https://cdn.cytivalifesciences.com/api/public/content/U5B2R9kFME-BRgtAeIRoWQ-pdf

Procedure: Covalent coupling procedures for Sera-Mag and Sera-Mag speedbeads carboxylate-modified magnetic particles

The following protocols outline the suggested materials and process for coupling of Sera-Mag and Sera-Mag speedbeads carboxylate-modified magnetic particles to proteins and oligonucleotides. All protocols can be used with Sera-Mag and Sera-Mag speedbeads products. These recommended coupling protocols are designed for:

- Optimal covalent coupling of proteins and oligonucleotides to our magnetic particles
- Covalent coupling of proteins using a choice of two protocols
- Simplicity, efficiency, and confidence

https://cdn.cytivalifesciences.com/api/public/content/digi-33629-pdf

Procedure: Sonication and mixing of Sera-Mag particles

Effective processing is one of the most critical aspects of magnetic particle utilization. Monodispersity and homogeneous suspension of particles should be carefully controlled during use to ensure robust and reproducible performance. This procedure discusses methods for successful use of Sera-Mag magnetic particle technology.

https://cdn.cytivalifesciences.com/api/public/content/9qxHxNN0R2WAJ8tZGB8l6Q-pdf?

Procedure: Sera-Mag speedbeads magnetic Protein A/G particles

Procedures for manual and automated antibody purification and manual immunoprecipitation.

https://cdn.cytivalifesciences.com/api/public/content/hXVG-kM0MEe1KOeOWUGoCQ-pdf

Procedure: Sera-Mag speedbeads streptavidin-blocked magnetic particles

Procedure for manual immunoprecipitation using a biotinylated antibody and troubleshooting tips.

https://cdn.cytivalifesciences.com/api/public/content/Vfip9mdbH0WOaEODfV0miw-pdf

04

Further information

Handbook: Total solutions for preparation of histidine-tagged proteins

Cytiva offers a wide range of products for convenient and flexible purification of histidine-tagged proteins produced in prokaryotic and eukaryotic expression systems with a choice of uncharged or nickel/cobalt precharged media. Use the workflow to locate the right product for your needs including high protein binding capacity with low leakage of nickel or cobalt ions and different formats allow screening, capture purification, and scale-up.

https://cdn.cytivalifesciences.com/api/public/content/digi-14022-pdf

Handbook: Protein sample preparation

This handbook provides an overview of protein sample preparation, a conceptual, high-level workflow for protein sample preparation and analysis, and parallel processing and screening strategies in recombinant protein and monoclonal antibody production workflows.

https://cdn.cytivalifesciences.com/api/public/content/digi-15794-pdf

E-learning: Get to know: working with magnetic beads

This free, interactive course highlights the key considerations when working with magnetic beads. From handling to elution techniques that can enhance binding, sensitivity, and specificity of an experiment and optimize results.

https://www.cytivalifesciences.com/en/us/training/training-catalog/object/course-5453668

Infographic: Mastering magnetic bead characteristics

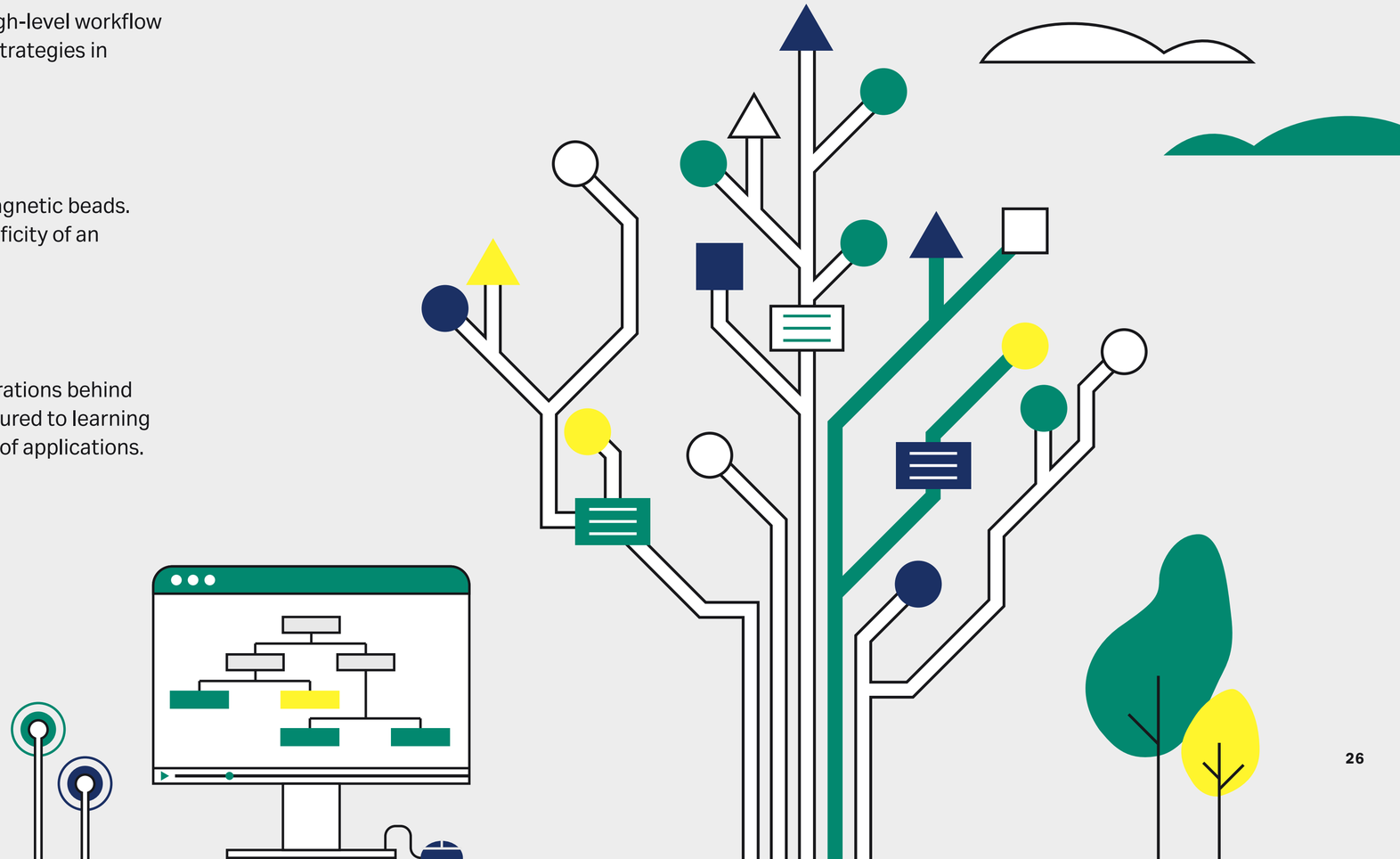
Download this comprehensive troubleshooting guide to unlock some of the considerations behind magnetic bead characteristics. From top tips on how these characteristics are measured to learning why each is important for optimal performance to enhance the efficiency of a range of applications.

https://info.cytivalifesciences.com/magnetic-bead-troubleshooting-guide.html

Infographic: Choosing the right magnetic bead

Based on what needs to be achieved and the attributes of the chosen target, the decision tree infographic helps the decision making process for the right magnetic bead to enhance and optimize binding, sensitivity and specificity of experiments.

https://info.cytivalifesciences.com/chem-infographic-mb-form.html



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