Purification Beads, Columns & Resins

SMALL-SCALE, HIGH-THROUGHPUT APPLICATIONS AND LARGE SCALE PURIFICATION STRATEGIES



Introduction

Isolation of pure substrates or proteins for downstream experiments is a common, yet time consuming, task. New England Biolabs offers a variety of resins and magnetic beads that are easy-to-use, highly specific, and available in several different formats for rapid isolation and purification of proteins, nucleic acids and immunoglobulins. NEB's magnetic beads are ideally suited for applications involving high-throughput proteomic screening, small-scale protein isolation, immunomagnetic isolations or cell separation experiments. With magnetic beads, affinity purification of tagged proteins, antigens, antibodies and nucleic acids can be done conveniently and quickly. Immobilized substrates remain biologically active and can be eluted in small volumes or serve as ligands in subsequent pull-down or target interaction experiments involving DNA or proteins. NEB's resins enable simple, one-step purification strategies for tagged proteins, and result in a high yield of highly pure substrate. For the full list of products available for protein expression and purification, visit www.neb.com/ProteinExpression.

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	PROTEIN PURIFICATION	LARGE-SCALE Purifications	USE IN AUTOMATED Chromatography	HIGH- Throughput	BIOTINYLATED Substrate binding	PROTEIN Pull-down	NUCLEIC ACID Pull-down	mRNA PURIFICATION/ Pull-down	IMMUNOPRECIPITATION	CELL SEPARATION/ CELL SORTING
NEBExpress™ Ni-NTA Magnetic Beads (NEB #S1423)	● (His-tag)			٠		•				
NEBExpress Ni Spin Columns (NEB #S1427)	(His-tag)			•		•				
NEBExpress Ni Resin (NEB #S1428)	(His-tag)	٠	•			•				
Amylose Resin (NEB #E8021)	(MBP)	•				•				
Amylose Resin High Flow (NEB #E8022)	(MBP)	•	•			•				
Amylose Magnetic Beads (NEB #E8035)	(MBP)			•		•				
Anti-MBP Magnetic Beads (NEB #E8037)	(MBP)			•		•				
Chitin Resin (NEB #S6651)	• (intein-CBD tag)	•				•				
Chitin Magnetic Beads (NEB #E8036)	• (intein-CBD tag)			٠		•				
Oligo d(T) ₂₅ Magnetic Beads (NEB #S1419)				•			•	•		
Streptavidin Magnetic Beads (NEB #S1420)				٠	•	(biotinylated bait)	(biotinylated bait)			
Hydrophilic Streptavidin Magnetic Beads (NEB #S1421)				•	•	(biotinylated bait)	(biotinylated bait)			
Protein A Magnetic Beads (NEB #S1425)				•					٠	
Protein G Magnetic Beads (NEB #S1430)				٠					٠	
Goat Anti-Mouse IgG Magnetic Beads (NEB #S1431)				•					(Mouse IgGs)	•
Goat Anti-Rabbit IgG Magnetic Beads (NEB #S1432)				٠					(Rabbit IgGs)	•
Goat Anti-Rat IgG Magnetic Beads (NEB #S1433)				٠					(Rat IgGs)	•
Magnetic mRNA Isolation Kit (NEB #S1550)				٠				٠		

Product Selection Chart

Polyhistidine-tagged Protein Purification

NEBExpress[™] Ni-NTA Magnetic Beads

NEBExpress Ni-NTA magnetic beads are an affinity matrix for the small-scale isolation and purification of polyhistidine-tagged (His-tagged) fusion proteins in manual or automated formats. They are prepared with agarose-based, super-paramagnetic microparticles that provide high binding capacity and fast magnetic response. Immobilized Metal Affinity Chromatography (IMAC) purification employing NEBExpress Ni-NTA magnetic beads can be performed under native or denaturing conditions, thereby allowing efficient binding and purification of insoluble proteins, proteins that aggregate in inclusion bodies, or proteins with tertiary structures that occlude the polyhistidine affinity tag. Low non-specific binding properties permit immobilized fusion proteins to be used in reverse purification schemes or in subsequent interaction experiments to capture or pull-down protein complexes from crude cell lysates. Additionally, these beads enable screening of expression and purification conditions to streamline functional and structural characterization of target proteins.

Binding Capacity:

Varies with target, typically \geq 7.5 mg His-tagged fusion protein/ml bed volume

Ordering Information:

NEBExpress Ni-NTA Magnetic Beads (NEB #S1423S/L) 1/5 ml

NEBExpress Ni Resin

NEBExpress Ni Resin is an affinity matrix for the isolation and purification of polyhistidine-tagged (His-tagged) fusion proteins. It is intended for use in gravity or pressure flow columns and batch purifications. NEBExpress Ni Resin is comprised of a highly uniform and chemical-tolerant resin that is pre-charged with nickel ions on the matrix surface. It is resistant to a wide range of chemicals, including NaOH, EDTA, and commonly used reducing agents such as TCEP, DTT, and β -mercaptoethanol.

Binding Capacity:

1 ml of NEBExpress Ni Resin will bind \geq 10 mg of His-tagged fusion protein

Ordering Information:

NEBExpress Ni Resin (NEB #S1428S)	ml
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Companion Product:

TEV Protease

A highly-specific cysteine protease that is ideal for removal of affinity tags, such as maltose binding protein (MBP) or poly-histidine (His-tag) from fusion proteins.

Ordering Information:

TEV Protease (NEB #P8112S)1,000 units

FEATURES

- Suitable for high-throughput and scalable purification strategies
- High specific binding yields purities of > 95% in a singlepurification step
- Nitrilotriacetic acid (NTA) coordination exhibits low nickel ion leaching
- Tolerates a wide range of conditions, including the presence of protein denaturants and detergents. Compatible with commercially available detergent-based cell lysis reagents
- Elution can be achieved by protonation, ligand exchange (with imidazole) or extraction of the metal ion by a strong chelator (e.g., EDTA)
- · Includes all required buffers for purification

- Intended for use in gravity or pressure flow columns and batch purifications
- High specific binding yields purities of > 95% in a single-purification step
- Strong nickel ion binding provides excellent resistance to EDTA and reducing agents. Compatible with commercially available detergent-based cell lysis reagents.
- Can be used for isolation and purification of His-tagged fusion proteins under native or denaturing conditions

NEBExpress Ni Spin Columns

NEBExpress Ni Spin columns are pre-packed with agarose-based microparticles ranging in size from $10-100 \ \mu m$ for the small-scale isolation and purification of polyhistidine-tagged (His-tagged) fusion proteins. Immobilized Metal Affinity Chromatography (IMAC) purification employing NEBExpress Ni Spin columns can be performed under native or denaturing conditions, including conditions in which EDTA or reducing reagents are required, yielding highly pure target protein in a single purification step. This enables screening of expression conditions and streamlines the functional and structural characterization of the target protein.

Binding Capacity:

Varies with target, ≥ 1 mg His-tagged fusion protein per column

Ordering Information:

NEBExpress Ni Spin Columns (NEB #S1427S/L).....10/25 columns

FEATURES

- Includes ready-to-use pre-packed Ni spin columns and all required buffers for purification
- Purify \geq 1 mg His-tagged protein per column in as little as 15 minutes
- High specific binding yields purities of > 95% in a single purification step
- Strong nickel ion binding provides excellent resistance to EDTA, NaOH and reducing agents such as DTT and β-mercaptoethanol. Compatible with commercially available detergent-based cell lysis reagents.



NEBExpress Ni Spin Column Quick Start Protocol

Maltose Binding Protein (MBP) Purification

Amylose Resin

Amylose resin is an affinity matrix used for the isolation of proteins fused to maltosebinding protein (MBP). It is intended for use in a gravity flow column.

Binding Capacity: > 4 mg MBP5*-paramyosin Δ Sal fusion protein/ml amylose resin

Ordering Information:

Amylose Resin	(NEB #E8021S	/L)	
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FEATURES

- Highly specific binding of protein fused to MBP allows for one-step purification
- Easily construct MBP-fusion proteins using the NEBExpress MBP Fusion & Purification System (NEB #E8201)
- MBP is easily removed by Factor Xa Protease (NEB #P8010) or TEV Protease (NEB #P8112)
- · Ideal for use in gravity flow column
- · Can be regenerated and used multiple times

Amylose Resin High Flow

Amylose Resin High Flow is a cross-linked affinity matrix used for the isolation of proteins fused to maltose-binding protein (MBP). This cross-linked, rigid matrix can be used in automated chromatography systems.

Binding Capacity:

> 4 mg MBP5*-paramyosin \Dal fusion protein/ml amylose resin high flow

Ordering Information:

Amylose Resin High Flow (NEB #E8022S/L)..... 15/100 ml

FEATURES

- Highly specific binding of protein fused to MBP allows for one-step purification
- Easily construct MBP-fusion proteins using the NEBExpress MBP Fusion & Purification System (NEB #E8201)
- MBP is easily removed by Factor Xa Protease (NEB #P8010) or TEV Protease (NEB #P8112)
- · Ideal for use in automated chromatography systems
- · Can be regenerated and used multiple times

Amylose Magnetic Beads

An affinity matrix for the small-scale isolation and purification of maltose-binding protein (MBP) fusion proteins. Amylose is covalently coupled to a superparamagnetic particle through a linkage that is stable and cleavage resistant over a wide pH range.

Binding Capacity:

10 μg MBP5*-paramyosin ΔSal fusion protein/mg amylose magnetic beads

Ordering Information:

- Easily construct MBP-fusion proteins using the NEBExpress MBP Fusion & Purification System (NEB #E8201)
- Quick, small-scale purification of MBP-fusion proteins affords higher efficiency and enables high-throughput workflows
- Immobilized fusion proteins can be used in subsequent experiments to capture (pull down) target proteins from crude cell lysates

Anti-MBP Magnetic Beads

An affinity matrix for the small-scale isolation and purification of maltose-binding protein (MBP) fusion proteins. Monoclonal anti-MBP is covalently coupled to 1 μ m nonporous superparamagnetic particles through a linkage that is stable and cleavage resistant over a wide pH range, thereby permitting the immunomagnetic isolation of MBP-fusion proteins from cell culture extracts.

Binding Capacity:

5 μg MBP5*-paramyosin ΔSal fusion protein/mg Anti-MBP magnetic beads

Ordering Information:

Anti-MBP Monoclonal Antibody

Anti-MBP Monoclonal Antibody is a murine anti-maltose binding protein (MBP) antibody, isotype IgG2a. This antibody enables highly sensitive detection of nanogram levels of MBP-fused proteins when used with a fluorophore-labeled Anti-Mouse IgG secondary antibody.

Recommended Dilution:

1:10,000

Ordering Information:

Anti-MBP Monoclonal Antibody (NEB #E8032S/L).....0.05/0.25 ml

FEATURES

 Immobilized fusion proteins can be used in subsequent experiments to capture (pull down) target proteins that specifically interact with the immobilized MBP-fusion protein

- High purity and specificity for MBP-tag
- · Verified for use in both Western blotting and ELISA

Chitin Binding Domain (CBD) Purification

Chitin Resin

An affinity matrix for the isolation of target proteins fused to an intein-chitin binding domain (CBD).

Binding Capacity:

2~mg maltose-binding protein (MBP)/mL bed volume released from the resin after cleavage of the MBP-CBD-fusion

Ordering Information:

Chitin Resin (NEB #S6651S/L)20	/100 ml
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FEATURES

- CBD-fusion proteins can be easily constructed using the IMPACT Purification System (NEB #E6901)
- Strong specific binding for CBD-fusion protein enables purification of highly pure protein from crude lysate in one step
- Removal of CBD-tag during elution typically yields highly pure, native protein without the use of a protease
- This resin, when used with the pTXB1 Vector (NEB #N6707), allows for the isolation of native recombinant proteins possessing a reactive C-terminal thioester that can be used for applications in intein-mediated protein ligation (IPL) and sitespecific labeling
- · Resin may be regenerated up to 5 times

Chitin Magnetic Beads

An affinity matrix for the small-scale isolation of target proteins fused to a chitin binding domain (CBD). Chitin beads have been prepared with encapsulated magnetite, thereby permitting the magnetic isolation of CBD-fusion proteins from cell culture supernatants.

Binding Capacity:

2 mg of CBD-fusion protein/ml bed volume

Ordering Information:

FEATURES

- CBD-fusion proteins can be easily constructed using the IMPACT Purification System (NEB #E6901)
- Immobilized fusion proteins can be used in subsequent experiments to capture (pull down) target proteins from crude cell lysates
- Quick, small-scale purification of CBD-fusion proteins enables higher throughput and efficiency
- Removal of CBD-tag during elution typically yields highly pure, native protein
- The matrix can be regenerated

Anti-CBD Monoclonal Antibody

Anti-CBD Monoclonal Antibody is a murine anti-chitin binding domain (CBD) antibody, isotype IgG1.

Recommended Dilution:

1:1,000

Ordering Information:

- High purity and specificity for chitin binding domain tag
- Verified for use in both Western blotting and ELISA

SNAP-Tag[®] Purification

SNAP-Capture Pull Down Resin

An affinity matrix for the isolation of target proteins fused to a SNAP-tag. SNAP-Capture Pull Down Resin is an agarose-based resin used to selectively capture and immobilize a SNAP-tag fusion protein from solution. This resin consists of benzylguanine, a SNAP-tag binding substrate, covalently attached to highly cross-linked agarose (4%).

Binding Capacity:

1 mg of SNAP-tag fusion protein/ml bed volume

Ordering Information:

FEATURES

- · Extremely low non-specific binding of proteins from lysates
- · Ideal for pull-down applications
- SNAP-tag fusion proteins can be labeled with SNAP-tag substrates, including fluorophores and biotin

SNAP-Capture Magnetic Beads

An affinity matrix for the small-scale isolation of target proteins fused to a SNAP-tag. SNAP-Capture Magnetic Beads are used to selectively immobilize and magnetically separate a SNAP-tag fusion protein from solution using magnetic agarose beads. They are prepared by the coupling of SNAP-tag substrate benzylguanine with highly stable 20-100 μ m superparamagnetic particles.

Binding Capacity:

≥ 1 mg of target SNAP-tag fusion protein per ml of bead bed volume

Ordering Information:

FEATURES

- · Extremely low non-specific binding of proteins from lysates
- Quick, small-scale purification of SNAP-tag fusion proteins enables higher throughput and efficiency
- · Ideal for pull-down applications
- SNAP-tag fusion proteins can be labeled with SNAP-tag substrates, including fluorophores and biotin

Magnetic Bead Purification Products

Oligo d(T)₂₅ Magnetic Beads

Oligo $d(T)_{25}$ Magnetic Beads consist of oligo $d(T)_{25}$ covalently coupled to 1 µm superparamagnetic particle through a linkage that is stable over a wide pH range. These beads enable small-scale isolations of mRNA from a variety of samples, including *in vitro* transcribed mRNA, total RNA, crude cell lysates and tissue. The selectivity for mRNA results from the annealing of bead-linked oligo $d(T)_{25}$ to the poly(A) region present in most eukaryotic mRNAs.

Binding Capacity: $\geq 5 \ \mu g \ rA_{30}$ per mg of beads

Ordering Information:

- Simplify transcriptomics workflows by eliminating total RNA isolation steps and simply isolating mRNA from crude samples
- The magnetic separation technology is scalable and permits elution of intact mRNA in small volumes, thereby eliminating the need to precipitate isolated mRNA
- Covalent linkage of oligo $d(T)_{25}$ to the beads allows them to be reused, thereby enabling multiple isolations from the same sample input
- Elution of isolated mRNA is optional; the bound $d(T)_{\rm 25}$ can be used as a primer for reverse transcriptase in first-strand cDNA reactions

Magnetic mRNA Isolation Kit

The Magnetic mRNA Isolation Kit is designed to isolate intact poly(A)+ RNA from cells and tissue without requiring phenol or other organic solvents. The technology is based on the coupling of Oligo $d(T)_{25}$ to 1 µm paramagnetic beads, which is then used as the solid support for the direct binding of poly(A)+ RNA.

Ordering Information:

FEATURES

- Can be used for manual processing of multiple samples or automated for high-throughput applications
- Magnetic separation technology enables elution of intact mRNA in small volumes, eliminating the need for precipitating the poly(A)+ transcripts in the eluent
- · Intact poly(A)+ RNA isolated in less than one hour
- Oligo d(T)₂₅ Magnetic Beads can be reused up to three times with the same sample input

Streptavidin Magnetic Beads

Streptavidin Magnetic Beads are 1 μ m superparamagnetic particles covalently coupled to a highly pure form of streptavidin. The beads provide fast magnetic response times and reaction kinetics, and they have high binding capacity and sensitivity while retaining their physical integrity. They can be used to capture biotin-labeled substrates including DNA, RNA, peptides, antigens, antibodies and other proteins of interest in manual or automated workflows. These beads typically exhibit lower non-specific binding of proteins.

Binding Capacity:

 \ge 30 µg biotinylated antibody per mg of beads or > 500 pmol of single-stranded 25 bp biotinylated oligonucleotide per mg of beads

Ordering Information:

Streptavidin Magnetic Beads (NEB #S1420S)

Hydrophilic Streptavidin Magnetic Beads

Hydrophilic Streptavidin Magnetic Beads are $2-3 \ \mu m$ superparamagnetic particles covalently coupled to a highly pure form of streptavidin. The beads provide rapid magnetic response times and reaction kinetics, and they have high binding capacity and sensitivity while retaining their physical integrity. They can be used to capture biotin-labeled substrates including DNA, RNA, peptides, antigens, antibodies and other proteins of interest in manual or automated workflows. These beads typically exhibit lower non-specific binding of nucleic acids.

Binding Capacity:

> 400 pmol of single-stranded 25 bp biotinylated oligonucleotide per mg of beads

Ordering Information:

FEATURES

- Strong biotin-streptavidin interaction (Ka = 10¹⁵ M⁻¹), coupled with low, non-specific binding of streptavidin, permits captured substrates to be useful as ligands in sample preparation, nucleic acid isolation, immunoprecipitations and proteomics workflows
- Can be used for solution-phase panning in phage display experiments, SELEX, purification of DNA/RNA binding proteins and cell-based screening
- · Provided in an RNase-free solution

- Strong biotin-streptavidin interaction (Ka = 10¹⁵ M⁻¹), coupled with low, non-specific binding of streptavidin, permits captured substrates to be useful as ligands in sample preparation, nucleic acid isolation, immunoprecipitations and proteomics workflows
- Can be used for solution-phase panning in phage display experiments, SELEX, purification of DNA/RNA binding proteins and cell-based screening
- Provided in an RNase-free solution

Protein A and Protein G Magnetic Beads

Protein A Magnetic Beads are $2-3 \mu m$ superparamagnetic particles covalently coupled to a highly pure form of recombinant protein A. The beads allow for isolation of most mammalian immunoglobulins (IgGs) and are amenable to immunoprecipitation. Predominant Fc-binding allows optimal IgG orientation upon binding to the outer surface of the Protein A Magnetic Beads allowing Fab regions to efficiently bind antigen.

Protein G Magnetic Beads are $2-3 \mu m$ superparamagnetic particles covalently coupled to a highly pure form of recombinant protein G. The beads allow for isolation of most mammalian immunoglobulins (IgGs) and are amenable to immunoprecipitation. Predominant Fc-binding allows optimal IgG orientation upon binding to the outer surface of the Protein G Magnetic Beads allowing Fab regions to efficiently bind antigen.

These beads can be used to immunoprecipitate target proteins from crude cell lysates using a selected primary antibody. In addition, specific antibodies can be chemically cross-linked to the Protein A- or Protein G- coated surface to create a reusable immunoprecipitation bead, thereby avoiding the co-elution of antibody with the target antigen.

Binding Capacity:

 $> 280 \ \mu g$ of Human IgG per ml of beads

Ordering Information:

Protein A Magnetic Beads (NEB #S1425S)	ıl
Protein G Magnetic Beads (NEB #S1430S)	ıl

Goat Anti-Mouse IgG Magnetic Beads

An affinity matrix for the small-scale immunomagnetic separation and purification of mouse IgG. Specifically, the beads consist of Anti-Mouse IgG that is covalently coupled to a 1 μ m nonporous superparamagnetic particle.

Binding Capacity:

5 μ g mouse lgG/mg Goat Anti-Mouse IgG Beads

Ordering Information:

Goat Anti-Mouse IgG Magnetic Beads (NEB #S1431S)......20 mg

FEATURES

- Exhibits high affinity for subclasses of IgG from many species, including human, rabbit, mouse, rat and sheep
- Protein G can be used for immuno-precipitations with mouse monoclonal antibodies
- Protein coupling is stable and cleavage resistant over a wide pH range. This permits the immunomagnetic purification of IgGs from ascites, serum or cell culture supernatants
- · Can be regenerated without loss of binding capacity
- Quick, small-scale purification of many subclasses of IgG affords higher throughput and efficiency

FEATURES

- This secondary antibody binds the heavy chain of mouse IgG and is suitable for immunoassays that employ a mouse IgG primary monoclonal antibody
- Cell separations and sorting can be accomplished using a mouse IgG antibody to defined cell surface antigens
- Quick, small-scale purification of mouse IgG affords higher throughput and efficiency

Goat Anti-Rabbit IgG Magnetic Beads

An affinity matrix for the small-scale immunomagnetic separation and purification of rabbit IgG. Specifically, the beads consist of Goat Anti-Rabbit IgG that is covalently coupled to a 1 μ m nonporous superparamagnetic particle.

Binding Capacity:

5 µg rabbit lgG/mg Goat Anti-Rabbit IgG Magnetic Beads

Ordering Information:

Goat Anti-Rabb IgG Magnetic Beads (NEB #S1432S) 1 ml
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- This secondary antibody binds the heavy chain of all rabbit IgG subclasses and is suitable for immunoassays that employ a rabbit IgG primary polyclonal antibody
- Cell separations and sorting can be accomplished using a rabbit IgG antibody to defined cell surface antigens
- Quick, small-scale purification of rabbit IgG affords higher throughput and efficiency

Goat Anti-Rat IgG Magnetic Beads

An affinity matrix for the small-scale immunomagnetic separation and purification of rat IgG. Specifically, the beads consist of anti-Rat IgG that is covalently coupled to a 1 μ m nonporous superparamagnetic particle.

Binding Capacity:

5 µg rat IgG/mg of Goat Anti-Rat IgG Magnetic Beads

Ordering Information:

Goat Anti-Rat IgG Magnetic Beads (NEB #S1433S)	1 ml
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FEATURES

- This secondary antibody binds the Fc portion of all monoclonal rat IgG subclasses and is suitable for immunoassays that employ a rat IgG primary monoclonal antibody
- Cell separations and sorting can be accomplished using a rat IgG antibody to defined cell surface antigens
- Quick, small-scale purification of rat IgG affords higher throughput and efficiency

Magnetic Separation Racks

	APPLICATION	MAGNETS	CAPACITY	CONVENIENCE
6-Tube Magnetic Separation Rack (NEB #S1506)	Designed for small-scale separations using magnetic particles	Neodymium rare earth permanent magnets	6 tubes (1.5 ml)	Use with magnetic particle-based affinity purification for rapid, small-scale purifications
50 ml Magnetic Separation Rack (NEB #S1507)	Designed for small-scale separations using magnetic particles	Neodymium rare earth permanent magnets	4 tubes (50 ml)	Use with magnetic particle-based affinity purification for rapid, streamlined purifications
<u>12-Tube Magnetic</u> <u>Separation Rack</u> (NEB #S1509)	Designed for small-scale separations using magnetic particles	Neodymium rare earth permanent magnets	12 tubes (1.5 ml)	Use with magnetic particle-based affinity purification for rapid, small-scale purifications
96-Well Microtiter Plate Magnetic Separation Rack (NEB #S1511)	Designed for use with commercially available high-flanged 100 µl to 300 µl flat-bottom 96-well microplates	24 side-pull magnetic pins attract magnetic beads from solution to the side walls of four adjacent wells	96-well	The orientation of the magnetic field ensures complete removal of the magnetic beads from solution during pipetting steps, thereby minimizing sample loss
NEBNext [®] Magnetic Separation Rack (NEB #S1515)	Designed for rapid and effective small- scale separations of magnetic particles	Anodized aluminum rack with Neodymium Iron Boron (NdFeB) rare earth magnets	24 tubes (0.2 ml)	Next generation sequencing library preparation workflows include magnetic bead-based purification and size-selection steps. It is important for library yield and quality that bead separation be highly efficient and fast, and this is enabled by the powerful fixed magnet cores in this rack.

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