Cell isolation

Dynabeads magnetic beads

Gentle and reliable cell isolation

invitrogen

Cell isolation

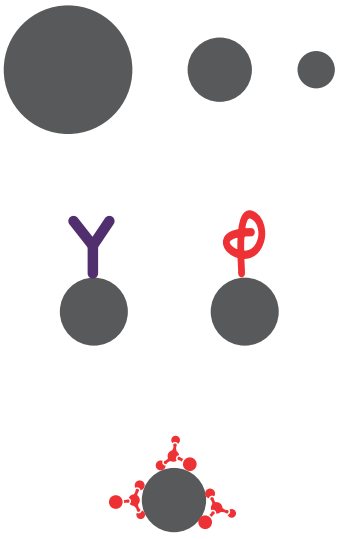
For 40 years, Invitrogen™ Dynabeads™ magnetic beads have been at the forefront of cell isolation technology. These superparamagnetic beads are renowned for their consistent physical and chemical properties (e.g., uniform and monodisperse), which make them indispensable for a variety of applications. Dynabeads magnetic beads enable high-performance, gentle isolation of immune cells and other cell types. Dynabeads magnetic beads can also facilitate isolation of viable cells from a variety of species with the cell separation method that best suits your research needs.

Exceptional reproducibility and quality with all-species versatility

Dynabeads magnetic bead technology

With Dynabeads magnetic beads, you can specifically isolate cells from suspensions of human, mouse, and other types of cells, by positive isolation, negative isolation, or depletion. The gentle magnetic separation process enables high yields of pure, viable, and functional cells that retain their native characteristics. No columns are required for efficient magnetic separation. Because Dynabeads magnetic beads are not made from biodegradable materials, they are highly stable with a minimum shelf life of 18 months, and most have a shelf life of up to 3 years.

There are two main considerations for Dynabeads products: bead size and coating. Many optimized Dynabeads magnetic beads are available for immediate use in a variety of applications. Alternatively, you can easily prepare the beads with your own antibody, oligonucleotide, or other ligand of choice.



Size: Dynabeads magnetic beads come in 4.5 µm, 2.8 µm, and 1.0 µm sizes for different applications. Larger beads are typically used for isolation and expansion of cells. Smaller beads are used to isolate molecules, including proteins and nucleic acids.

Specificity: Dynabeads pre-coated with antibodies against cell surface and vesicles markers are available for immediate use. Custom specificity can be accommodated using secondary-coated or surface-activated beads.

Flexibility: Secondary-coated and surface-activated Dynabeads offer high flexibility for designing magnetic beads with the specificity of choice. Examples of secondary-coated Dynabeads specificities are Proteins A and G, streptavidin, anti-mouse, and anti-rat.

Automation compatibility with KingFisher instruments

Automation compatibility

Dynabeads magnetic bead–based separation protocols are developed to be simple, efficient, and gentle on cells. No centrifugation steps or columns are required for sample processing, and the protocols can be automated on Thermo Scientific™ KingFisher™ systems. Figure 1 shows an example of a fully automatable cell isolation workflow.

The synergy between Dynabeads magnetic beads and KingFisher instruments facilitates successful cell isolation integrated with protein isolation and analysis. Best of all, the time-to-results can be complete in a matter of hours.

Example workflow

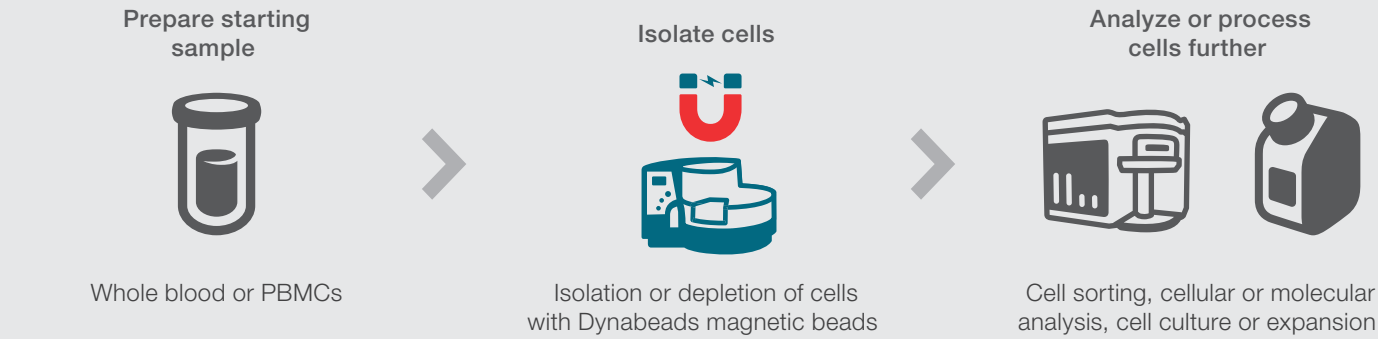


Figure 1. A variety of Dynabeads magnetic beads have KingFisher instrument automation scripts that allow isolation of 1–96 samples in a single run.

Automated cell and protein isolation using Dynabeads magnetic beads and KingFisher instrumentation

In an application note, we highlight an automated approach to cell isolation integrated with protein isolation using Dynabeads magnetic beads and the Thermo Scientific™ KingFisher™ Flex,* KingFisher™ Duo Prime, and KingFisher™ Apex* purification systems. This automated approach streamlines the workflow by combining cell isolation with subsequent protein isolation, reducing the amount of time required for immunoprecipitation and protein analysis to a single workday. The workflow minimizes hands-on steps to enhance efficiency and reproducibility, exemplified by positive isolation of T cells, monocytes, and B cells from peripheral blood mononuclear cells (PBMCs) (Figure 2).

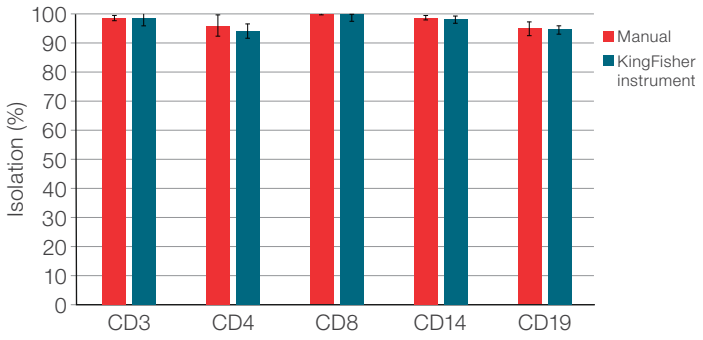


Figure 2. Efficiency of isolation of T cell subclasses (CD3, CD4, CD8), monocytes (CD14), and B cells (CD19) from PBMCs. Invitrogen™ Dynabeads™ CD3, Dynabeads™ CD4, Dynabeads™ CD8, Dynabeads™ CD14, and Dynabeads™ CD19 Pan B magnetic beads were incubated with PBMCs for 30 minutes using a KingFisher instrument. Isolation efficiency was compared to the manual approach using flow cytometry.

* For Laboratory Use.

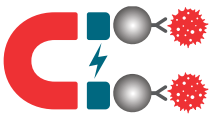
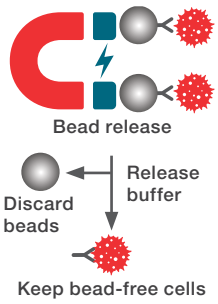
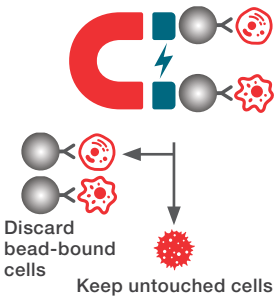
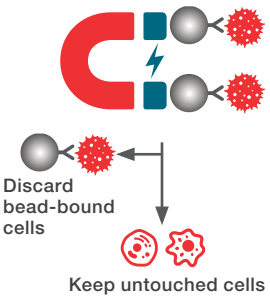
Cell isolation methods

Isolation of cells with Dynabeads magnetic beads can be performed using three main techniques—positive isolation, negative isolation, and cell depletion—or combinations thereof. Positive isolation can further be divided into positive isolation without bead release (resulting in bead-bound cells of interest) and positive isolation with bead release (resulting in bead-free cells of interest). These methods are described in more detail here and in Table 1.

- **Positive isolation**—Positive isolation of cells is performed using magnetic beads coated with cell surface marker–specific binders (generally, specific antibodies). The isolated cell population is defined by expression of the surface markers targeted by the binders on the magnetic beads.
  - **Positive isolation without release**—The bead–cell complexes are separated from the sample matrix (e.g., unbound cells, soluble biomolecules, debris) using a magnet. The supernatant is discarded, and the bead-bound cells are resuspended in the desired buffer for downstream cell culture or molecular analysis.
  - **Positive isolation with release**—The bead–cell complexes are separated from the sample using a magnet, then washed and treated with a release buffer to separate the beads and cells. The washed beads are removed using a magnet, and the bead-free cells are available for any downstream application.
- **Negative isolation**—Negative cell isolation involves depletion of unwanted cell types from the sample, leaving the target cells untouched and free of antibodies and beads. An antibody mix specific to the unwanted cells is first added to the sample, followed by addition of magnetic beads that are specific for the labeling antibodies. The bead–cell complexes are removed using a magnet, leaving the pure and untouched cells of interest in the supernatant.
- **Cell depletion**—Cell depletion is performed using magnetic beads conjugated with a specific antibody for depletion of all cells expressing the corresponding cell surface molecule. The bead-bound cells are removed from the sample using a magnet. The depleted sample is less complex and enriched for the cells of interest. This method may be used for detection and analysis of rare cells or downstream flow sorting.

Table 1 illustrates the various methods of isolating cells with Dynabeads magnetic beads and provides an overview of the benefits associated with each. Potential applications of each method are also listed.

Table 1. Cell isolation methods with Dynabeads magnetic beads.

	Positive isolation		Negative isolation	Cell depletion
	without release	with release		
Method				
Use and benefits	Recommended for fast and easy isolation of cells from complex samples, like whole blood and tissue digests, for downstream molecular analysis when cell lysis is required. This method yields highly pure and unaltered cells.	Recommended for isolation of bead-free cells from complex samples, like whole blood and tissue digests, for downstream cell culture and flow analysis. This method yields very pure cells, due to the two-step process of selective bead binding and selective bead release.	Recommended for enrichment of untouched and bead-free target cells from PBMC samples. This isolation method has the least impact on cells.	Recommended for reducing sample complexity (e.g. CTC enrichment) or for efficient depletion of cells for better understanding of immune responses and disease mechanisms.
Applications	Molecular biology*	Flow cytometry, cell culture, molecular biology*	Flow cytometry, cell culture, molecular biology*	Flow cytometry of non-depleted fraction, cell culture, molecular biology,* CTC detection, immunological responses, disease mechanisms

* Genomics, proteomics, and transcriptomics

Cell isolation for downstream molecular applications

Dynabeads magnetic beads for positive isolation without release are well-suited for downstream applications that require cell lysis, such as molecular biology applications including genomics, proteomics, and transcriptomics. Other methods that provide bead-free target cells, such as cell depletion, can also be used for downstream molecular applications. Manual and KingFisher automated cell isolation methods are available to help provide user-friendly solutions with high flexibility (Figure 3). Dynabeads products that utilize these approaches are summarized in Table 2. Downstream molecular sample prep can also be included in the automated process using an Applied Biosystems™ MagMAX™ nucleic acid isolation kit on a KingFisher instrument. Find a kit for your application at **thermofisher.com/magmax**.

Key benefits:

- Consistently high purity, viability, and yield
- Compatibility with automated workflows using KingFisher instruments
- Highly reproducible and efficient cell isolation directly from whole blood samples

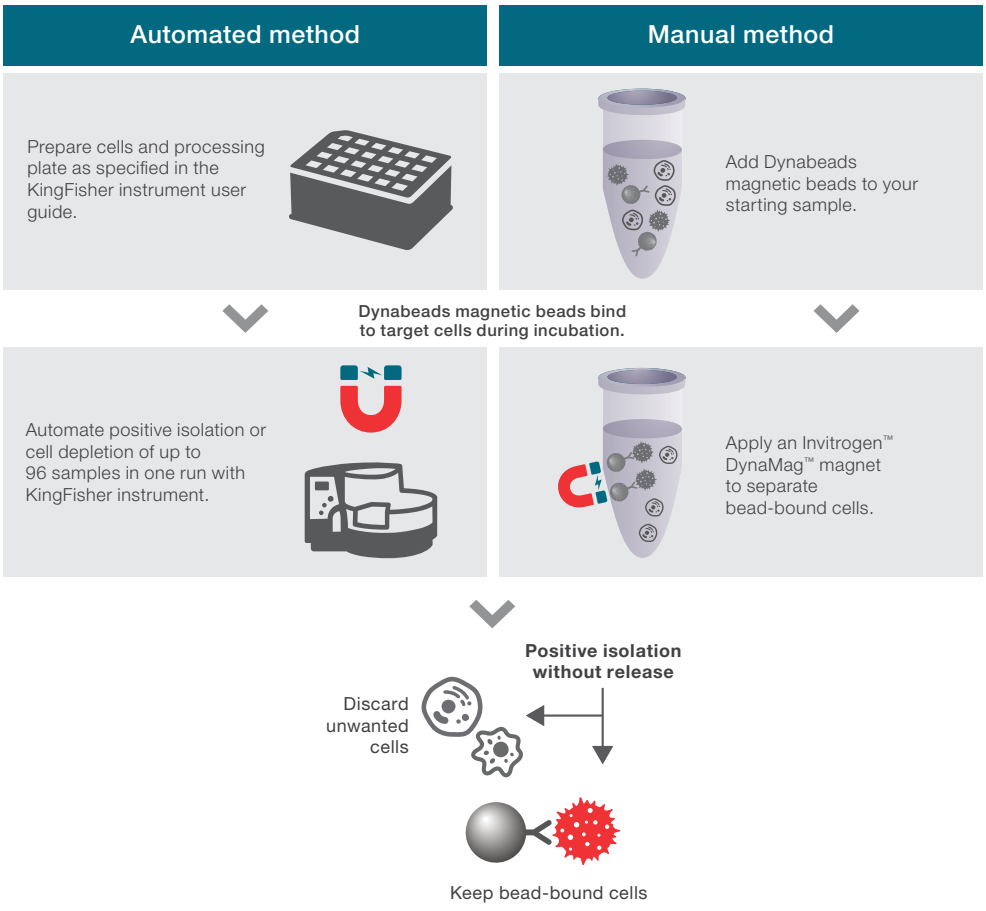


Figure 3. Manual and automated methods for cell isolation. Dynabeads magnetic beads for positive isolation without release are well-suited for downstream molecular applications such as genomics, proteomics, and transcriptomics.

Table 2. Pre-coated Dynabeads targeting specific cell populations, intended for positive isolation without release, or cell depletion.

Target cell species	Target cells	Cell surface markers	Sample type	Processing capacity	Recommended antibody	Suggested Invitrogen™ product	Automation scripts
Human	T cells and natural killer (NK) cells	CD2	PBMCs, blood	~2 x 10^9 cells total	Antibody covalantly coupled to the beads	Dynabeads™ CD2	
	Mature T cells	CD3	PBMCs, blood	~4 x 10^8 cells per mL of product		Dynabeads™ CD3	✓
	T helper cells	CD4	PBMCs, blood	~4 x 10^8 cells per mL of product		Dynabeads™ CD4	✓
	Cytotoxic T cells	CD8	PBMCs, blood	~4 x 10^8 cells per mL of product		Dynabeads™ CD8	✓
	Monocytes	CD14	PBMCs, blood	~4 x 10^8 cells per mL of product		Dynabeads™ CD14	✓
	Neutrophil and eosinophil granulocytes	CD15	PBMCs, blood	~4 x 10^8 cells per mL of product		Dynabeads™ CD15	✓
	Pan B cells	CD19	PBMCs, blood	~4 x 10^8 cells per mL of product		Dynabeads™ CD19 Pan B*	✓
	Regulatory T cells	CD25	PBMCs, blood	~5 x 10^9 cells total		Dynabeads™ CD25	
	Endothelial cells	CD31	Tissue digests	~2 x 10^9 cells total		Dynabeads™ CD31 Endothelial Cell**	
	Leukocytes	CD45	PBMCs, blood	~5 x 10^8 cells total		Dynabeads™ CD45	
			PBMCs, blood	~2.5 x 10^8 PBMCs per mL of product		Dynabeads™ MyOne™ CD45 Leukocyte Depletion	✓
	Epithelial cells	EpCAM	PBMCs, blood	~4 x 10^8 cells per mL of product		Dynabeads™ Epithelial Enrich	✓
Mouse	$CD4^+$ T cells	CD4	Lymph node, blood, spleen	~2 x 10^9 cells total		Dynabeads™ Mouse CD4	
	$CD8^+$ T cells	CD8	Lymph node, blood, spleen	~2 x 10^9 cells total		Dynabeads™ Mouse CD8 (Lyt 2)	
	Pan T cells	Thy1.2	Lymph node, blood, spleen	~2 x 10^9 cells total		Dynabeads™ Mouse Pan T (Thy1.2)	
	Pan B cells	B220	Lymph node, blood, spleen	~2 x 10^9 cells total		Dynabeads™ Mouse Pan B (B220)	

* For positive isolation and release of B cells, the Invitrogen™ DETACHaBEAD™ CD19 Kit needs to be used in combination with Dynabeads CD19 magnetic beads.

** Bead-bound cells can be cultured

Note: To build your own positive isolation or cell depletion product for custom targeting of cells from a wide range of species, see options on page 10.

Selected Dynabeads magnetic beads for cell isolation are now offered in 2 mL sizes for easy trial in your lab.

Cell isolation for downstream flow cytometry and cell culture applications

Dynabeads magnetic beads for positive isolation with release or negative isolation are ideal for downstream applications that require viable, bead-free cells, such as flow cytometry and most cell culture applications. Dynabeads magnetic beads pre-coated with antibodies for target cells, or beads to which you can add your own antibody, facilitate positive isolation with bead release options that allow the isolated cells to be used in any downstream application (Figure 4). Alternatively, Invitrogen™ Dynabeads™ Untouched™ kits for negative isolation contain optimized antibody mixes and beads for depletion of unwanted cells, which leaves target cells untouched and bead-free. These products are summarized in Tables 3 and 4. Dynabeads magnetic beads for cell depletion (Table 2) also leave the cells of interest bead-free and available for any downstream application. You can also build a custom kit with Dynabeads magnetic beads and your own antibody or antibody mix (see "Dynabeads magnetic beads for customized cell isolation" section).

Key benefits:

- Consistently high purity, viability, and yield
- High reproducibility
- Target cells remain bead-free

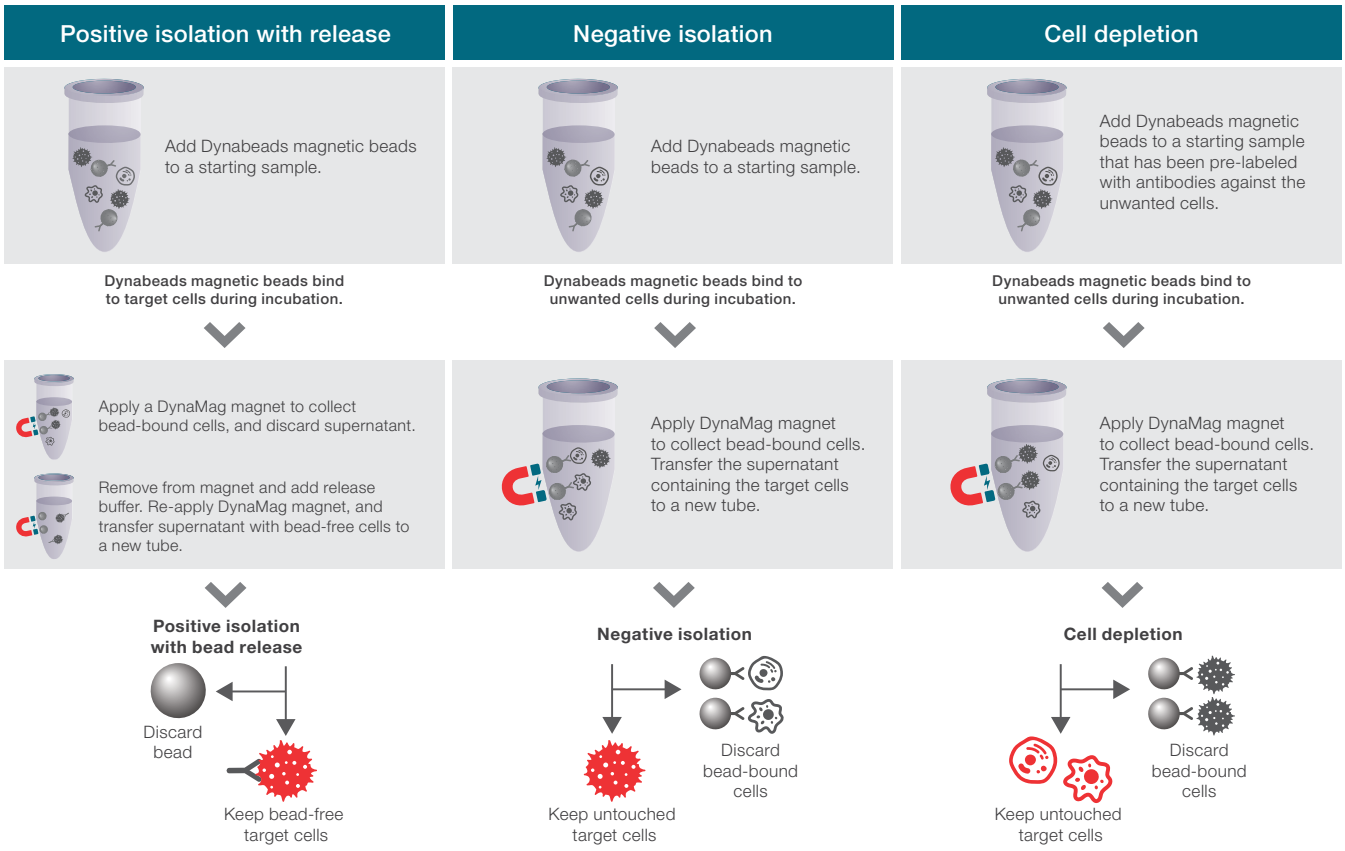


Figure 4. Use of Dynabeads magnetic beads for positive cell isolation with release, negative cell isolation, and cell depletion, for downstream flow cytometry and cell culture applications.

Table 3. Positive isolation with bead release for downstream flow cytometry and cell culture applications.

Target cell species	Target cells	Cell surface markers	Sample type	Processing capacity	Recommended antibody	Suggested Invitrogen™ product	Automation scripts
Any species	Any cell	Any marker	PBMCs, tissue digests, blood	~2 x 10^9 cells total	Biotinylated antibody	CELLection™ Biotin Binder Kit	
		Any marker	PBMCs, tissue digests, blood	~2 x 10^9 cells total	Any antibody; DSB-X biotinylation kit for the antibody is supplied	Dynabeads™ FlowComp™ Flexi Kit	
Any species (except mouse)		Any marker	PBMCs, tissue digests, blood	~2 x 10^9 cells total	Mouse IgG	CELLection™ Pan Mouse IgG Kit	
Human	T cells	CD3	MNCs, blood	80 mL whole blood, 2 x 10^9 PBMCs	Antibody provided in the kit	Dynabeads™ FlowComp™ Human CD3 Kit	✓
	T helper cells	CD4	PBMCs, blood	~2 x 10^9 cells total		Dynabeads™ FlowComp™ Human CD4 Kit	✓
	Cytotoxic T cells	CD8	PBMCs, blood	~2 x 10^9 cells total		Dynabeads™ FlowComp™ Human CD8 Kit	✓
	Monocytes	CD14	PBMCs, buffy coat, blood	2 x 10^9 PBMCs, 80 mL whole blood, 160 mL buffy coat		Dynabeads™ FlowComp™ Human CD14 Kit	
	Pan B cells	CD19	PBMCs, blood	1 x 10^7 PBMCs per isolation		DETACHaBEAD™ CD19 Kit*	
	Regulatory T cells	CD4/CD25	PBMCs	~1 x 10^9 cells total		Dynabeads™ Regulatory CD4+/CD25+ T Cell Kit	
	Tumor cells, epithelial cells	EpCAM	PBMCs, blood	~4 x 10^9 cells total		CELLection™ Epithelial Enrich Dynabeads™	
Mouse	$CD4^+$ T cells	CD4	Lymph node, blood, spleen	~2 x 10^9 cells total		Dynabeads™ FlowComp™ Mouse CD4 Kit	
	$CD8^+$ T cells	CD8	Lymph node, blood, spleen	~2 x 10^9 cells total		Dynabeads™ FlowComp™ Mouse CD8 Kit	

* For positive isolation and release of B cells, the DETACHaBEAD CD19 Kit needs to be used in combination with Dynabeads CD19 magnetic beads.

Table 4. Negative isolation for downstream flow cytometry and cell culture applications.

Target cell species	Target cells	Cell surface markers	Sample type	Processing capacity	Recommended antibody	Suggested Invitrogen™ product	Automation scripts
Human	Monocytes	CD3, CD7, CD16 (a and b), CD19, CD56, CD123, and CD235a	PBMCs, MNCs	~1 x 10^9 cells per kit	Optimized antibody mixes supplied in the kits	Dynabeads™ Untouched™ Human Monocytes Kit	
	Natural killer cells (NKs)	HLA Class II, CD3, CD14, CD36, CD123, and CD235a	PBMCs, MNCs	~1 x 10^9 cells per kit		Dynabeads™ Untouched™ Human NK Cells Kit	
	Pan B cells	CD2, CD14, CD16 (a and b), CD36, CD43, and CD235a	PBMCs, MNCs	~1 x 10^9 cells per kit		Dynabeads™ Untouched™ Human B Cells Kit	
	T cells (T helper cells, regulatory T cells, and natural killer T cells)	CD4	PBMCs	~1 x 10^9 cells per kit		Dynabeads™ Untouched™ Human CD4 T Cells Kit	
	Cytotoxic T cells	CD8	PBMCs	~1 x 10^9 cells per kit		Dynabeads™ Untouched™ Human CD8 T Cells Kit	
	Pan T cells (whole population)	CD14, CD16 (a and b), CD19, CD36, CD56, CD123, and CD235a	PBMCs	~1 x 10^9 cells total		Dynabeads™ Untouched™ Human T Cells Kit	
	Dendritic cells (DCs)	CD3, CD14, CD16, CD19, CD56, and CD235a	PBMCs	~2 x 10^9 cells total		Dynabeads™ Human DC Enrichment Kit	
Mouse	Pan B cells	CD43	Lymph node, tissue digests, spleen	~2 x 10^9 cells total		Dynabeads™ Mouse CD43 (Untouched™ B Cells)	
	$CD4^+$ T cells	CD4	Lymph node, MNCs, spleen	~5 x 10^7 cells per mL of product		Dynabeads™ Untouched™ Mouse CD4 Cells Kit	
	$CD8^+$ T cells	CD8	Lymph node, spleen	~1 x 10^9 cells total		Dynabeads™ Untouched™ Mouse CD8 Cells Kit	
	Pan T cells (whole population)	CD11b, CD16/32, CD45R, and Ter-119	Lymph node, spleen	~1 x 10^9 cells total		Dynabeads™ Untouched™ Mouse T Cells Kit	
	DCs	CD2, CD3ε, CD49b, mIgM, and Ter-119	Lymph node, spleen	~1 x 10^9 cells total		Dynabeads™ Mouse DC (Dendritic Cell) Enrichment Kit	

Dynabeads magnetic beads for customized cell isolation

Build your own positive isolation, negative isolation, or cell depletion product for a wide range of species with any antibody of your choice to isolate untouched or bead-bound target cells (Table 5).

Key benefits:

- All-species versatility
- Consistently high purity, viability, and yield
- High reproducibility

Table 5. Dynabeads magnetic beads for your own customized solution.

Type of isolation	Target cell species	Cell type targeted	Sample type	Processing capacity	Recommended antibody	Suggested Invitrogen™ product	Cat. No.	Automation scripts	Bead size
Positive isolation or cell depletion	Any species except mouse	Any cell of interest	PBMCs, tissue digests, blood	~2 x 10^9 cells total	Mouse IgG	Dynabeads™ Pan Mouse IgG	11040D (2 mL) 11041 (5 mL) 11042 (5 x 5 mL)	✓	4.5 µm
			PBMCs, tissue digests, blood	~2 x 10^9 cells total	Mouse IgG1, IgG2a, and IgG2b	Dynabeads™ Sheep Anti–Mouse IgG	11031 (5 mL)		4.5 µm
			PBMCs, tissue digests, blood	~2 x 10^9 cells total	Mouse IgG	Dynabeads™ Goat Anti–Mouse IgG	11033 (5 mL)		4.5 µm
			PBMCs, tissue digests, blood	~2 x 10^9 cells total	Mouse IgM	Dynabeads™ Rat Anti–Mouse IgM	11039D (5 mL)		4.5 µm
	Any species except rat		PBMCs, tissue digests, blood	~2 x 10^9 cells total	Rat IgG	Dynabeads™ Sheep Anti–Rat IgG	11036D (2 mL) 11035 (5 mL)	✓	4.5 µm
	Any species except rabbit		PBMCs, tissue digests, blood	~2 x 10^9 cells total	Rabbit IgG	Dynabeads™ M-280 Sheep Anti–Rabbit IgG	11203D (2 mL) 11204D (10 mL)	✓	2.8 µm
	Any species		PBMCs, tissue digests, blood	~2 x 10^9 cells total	Biotinylated antibody	Dynabeads™ Biotin Binder	11048D (2 mL) 11047 (5 mL)		2.8 µm
			Any sample type	Varies with application		Dynabeads™ MyOne™ Streptavidin T1	65601 (2 mL) 65602 (10 mL) 65604D (50 mL)		1 µm
Positive isolation with release			PBMCs, tissue digests, blood	~2 x 10^9 cells total	DSB-X biotinylated antibody; biotinylation kit supplied	Dynabeads™ FlowComp™ Flexi Kit	11061D (3 mL)		2.8 µm
Positive isolation or cell depletion	Any species	Any purified antibody or ligand	PBMCs, tissue digests, blood	~2 x 10^9 cells total	Any purified antibody or ligand	Dynabeads™ M-450 Epoxy			
			PBMCs, tissue digests, blood	~2 x 10^9 cells total		Dynabeads™ M-450 Tosylactivated			
			PBMCs, tissue digests, blood	~2 x 10^9 cells total		Dynabeads™ Antibody Coupling Kit†			

† The Invitrogen™ Dynabeads™ Antibody Coupling Kit contains buffers for optimal coupling conditions.

Commercial supply and OEM partnerships

We partner with leading biotech, healthcare, and therapeutics companies to provide Dynabeads magnetic beads that meet your business objectives, assuring you have the right beads, in the right format, and in the right quantities at the right time. We support partners from early assay development and validation through scale-up and commercialization.

Premium products: All Dynabeads products are analytically validated with a distinct level of reproducibility within and between batches. Patented manufacturing processes help ensure careful control of bead parameters and product properties, securing their performance.

Remarkable bead manufacturing: Our advanced manufacturing facilities can produce batches ranging from 1 g to 2 kg with close to zero off-grade batches, and our processes meet relevant regulatory and quality system compliance standards.

Comprehensive technical support: Our extensive proprietary knowledge enables customized product development, decreases quality control costs, and increases manufacturing efficiency. Hands-on support from R&D scientists is also available.

Find out more at **thermofisher.com/cellisolation**

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