Cancer research

Understanding cancer begins with the cell
Cancer research is an evolving field uncovering new mechanisms that drive tumor formation, growth, and metastasis. With advances in cancer research, Miltenyi Biotec continuously pushes the development of innovative products and technologies to meet the needs of this progressing field.

We offer optimized solutions for safe tumor tissue storage and gentle tumor tissue dissociation, preserving epitopes for downstream cell isolation and analysis.

Whether you are focusing on abundant tumor cells or less frequent infiltrating immune cells and tumor-associated fibroblasts in the tumor microenvironment, choose the suitable isolation kit and antibodies from our comprehensive portfolio.

Furthermore, we offer products for the generation of cell lines from primary tumors, the preparation of FFPE samples for tumor cell sorting and analysis, and the isolation and screening of exosomes.

Browse through this brochure to discover ideal solutions for every step of your specific cancer research workflow.
Cancer research workflow

Find the suitable entry point into our cancer research workflow depending on your starting material.

- **Sample preparation**
  - Get viable single-cell suspensions from solid tumors

- **Cell separation**
  - Efficiently isolate multiple cell populations or deplete unwanted cells from different tumor samples

- **Cell analysis**
  - Advance the analysis of your tumor samples

- **Cell culture**
  - Expand tumor cells from human primary or xenografted tissues

- **Exosome research solutions**
  - Find valuable kits for exosome isolation and analysis

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Start smart with the gentleMACS™ Portfolio

Everything you need for a fast, easy, and standardized tumor dissociation into single-cell suspensions:
- high yields of viable single cells from any tumor entity
- preservation of cell surface epitopes for reliable downstream analysis
- walk-away technology for minimal hands-on time

gentleMACS™ Dissociators
Fully or semi-automated operation for standardized sample preparation

Tissue Dissociation Kits
Optimized protocols and reagents for the dissociation of tumor tissue

gentleMACS™ Tubes
Engineered for efficient tissue dissociation or thorough cell homogenization

Strainers and filters
Removal of larger particles, cell clumps, or tissue fragments from complex cell suspensions

MACS® Tissue Storage Solution
Optimized storage of fresh tissue and tumor samples for up to 48 hours without activating cells or inducing apoptosis
From solid tumors to single cells

Tissue storage
Gain flexibility in your schedule with the MACS® Tissue Storage Solution. Your tumor samples are stored safely on their journey from collaboration sites or while you are busy processing other samples:
- optimized storage of tumor or healthy samples for up to 48 hours
- optimal cell viability and yield (fig. 1)
- no background effects, including cell activation or induction of apoptosis

Tumor dissociation
Using the gentleMACS™ Dissociators and Tissue Dissociation Kits, mechanical and enzymatic dissociation are combined to enable gentle, automated tumor dissociation for standardized and reproducible results.

Two types of uniquely designed gentleMACS Tubes with built-in rotors are used for all gentleMACS Dissociators. Choose the appropriate tube depending on your downstream application:
- gentleMACS C Tube (purple) to dissociate tissue and get viable single-cell suspensions
- gentleMACS M Tube (orange) to homogenize tissue for molecular applications

Preservation of epitopes is key
Epitopes are essential for numerous downstream applications, including cell isolation, cell sorting, and flow cytometry. Epitope preservation has been extensively tested after tissue dissociation using the gentleMACS Dissociators and Tumor Dissociation Kits:
- more than 200 epitopes tested, both in human and mouse
- preservation of sensitive epitopes by simple adjustment of the enzyme concentration

Figure 1: Comparison between MACS Tissue Storage Solution and competitor products (GMP-grade organ transplant solutions).
(A) Total cell yield in cells per gram of tissue. (B) Cell viability in percent.

Watch a short video on tumor dissociation and find our surface epitope preservation lists for download here: miltenyi-biotec.com/tumordissociation

For more detailed information please contact us.
Optimized dissociation protocols for solid tumors
Our Tumor Dissociation Kits combined with the suitable protocol are optimized for dissociation of solid tumors of different consistencies. The protocols allow to get high yields of viable single-cell suspensions, even from very small biopsy samples (fig. 2).

Prepare FFPE samples for tumor cell sorting and analysis
The formalin-fixed paraffin-embedded (FFPE) Tissue Dissociation Kit is optimized for the dissociation of human carcinoma sections (fig. 3). High yields of single cells can be obtained, while preserving important epitopes to distinguish cytokeratin-positive carcinoma cells and vimentin-positive non-carcinoma cells, e.g., fibroblasts.

Figure 2: Human melanoma metastases dissociated with the gentleMACS™ Dissociator. (A) Different cell types are visible in the single-cell suspension. TC: tumor cells, TIL: tumor-infiltrating leukocytes, ERY: erythrocytes. (B) Purity and viability after gating out red blood cells (RBCs).

Figure 3: Flow cytometry analysis of cytokeratin-positive and vimentin-positive cells after dissociation with the FFPE Tissue Dissociation Kit. Cells were labeled with fluorochrome-conjugated antibodies as indicated and analyzed before (A) and after sorting (B). Sorting resulted in two populations of cytokeratin-positive cells and vimentin-positive cells (B). Cells were analyzed using the MACSQuant® Analyzer 10.

Your results are only as good as your starting material.
Select the best with MACS® Technology

Efficient isolation of different target cell populations from tumor samples:
- fast and gentle isolation of viable and functional cells
- magnetic separation of cell populations based on surface antigens
- from manual separation to fully automated enrichment of 24 samples in parallel

**autoMACS® Pro Separator**
Fully automated cell separation for walk-away convenience

**MACS® Separator**
Fast and easy manual cell separation when used with MACS® Columns and MACS MicroBeads

**MultiMACSTM Cell24 Separator Plus**
Semi-automated cell separation of large sample numbers (for fully automated high-throughput cell separation refer to MultiMACSTM X)
Isolate your target cell population from tumors

Positive selection of tumor cells

Directly isolate tumor cells from your sample with MACS® Technology (fig. 4) using our MACS MicroBeads, REAlease® MicroBeads, or StraightFrom® MicroBeads. The sensitivity of MACS Technology allows for the isolation of tumor cells from dissociated solid and liquid tumors, as well as the isolation of circulating tumor cells (CTC) and disseminated tumor cells (DTC). If you are studying your own novel markers, you can simply use indirect magnetic labeling for customized cell separation.

Untouched tumor cell isolation

During untouched isolation, non-target cells are magnetically labeled and depleted (fig. 5). The Mouse Cell Depletion Kit, for example, uses a combination of antibodies to recognize all cells of mouse origin within xenograft tumors, regardless of tissue origin, leaving a pure population of human cells. Using the same separation principle, the Tumor Cell Isolation Kits enable the separation of human or mouse tumor cells by depleting all other cells (fig. 6).

Pure tumor cells for high-quality downstream analysis

- Cultivate tumor cells free of fibroblasts and other non-tumor cells.
- Increase sequencing accuracy and remove unspecified reads or cross-reactive probes.
- Benefit from more flexibility and channels for flow cytometry analysis.

For more information download our scientific poster here:

miltenyibiotec.com/tumor-kits
Isolation of tumor-associated fibroblasts

Tumor-associated fibroblasts play an important role in tumor development and progression. However, they may only account for about 0.5–5% of the total number of cells found in tumors. Applying a two-step strategy, tumor-associated fibroblasts are first pre-enriched by removing non-target cells, followed by isolation using the general fibroblast marker CD90.2 (fig. 7). This allows reliable isolation from different syngeneic mouse tumor models.

Isolation of TILs

The amount and composition of TILs is highly variable, complicating the analysis of individual subpopulations. When working with large cohort sizes, even immunophenotyping of TILs by flow cytometry is time consuming and data processing highly work intensive. Enrichment of TILs using CD45 (TIL) MicroBeads (fig. 8), CD4 (TIL) MicroBeads, CD8 (TIL) MicroBeads, or CD4/CD8 (TIL) MicroBeads (fig. 9) increases accuracy of analysis and significantly reduces the time needed for flow cytometry (tab. 1).

Table 1: Isolation of CD4⁺, CD8⁺, and pan T cells from different mouse tumor models using mouse CD4 (TIL) MicroBeads, CD8 (TIL) MicroBeads, and CD4/CD8 (TIL) MicroBeads dramatically decreases time of analysis.

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Cells to analyze</th>
<th>Events to collect</th>
<th>Flow cytometry time/sample*</th>
<th>Total flow cytometry time**</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4⁺ T cells</td>
<td>Bulk 5,000</td>
<td>796x10⁶</td>
<td>66.3 min</td>
<td>&gt;10 h</td>
</tr>
<tr>
<td>Isolated*** 5,000</td>
<td>5.41x10⁴</td>
<td>0.5 min</td>
<td>~11 min</td>
<td></td>
</tr>
<tr>
<td>CD8⁺ T cells</td>
<td>Bulk 5,000</td>
<td>2.80x10⁷</td>
<td>23.3 min</td>
<td>~3.5 h</td>
</tr>
<tr>
<td>Isolated*** 5,000</td>
<td>4.37x10⁴</td>
<td>0.4 min</td>
<td>~10 min</td>
<td></td>
</tr>
<tr>
<td>T cells</td>
<td>Bulk 10,000</td>
<td>8.13x10⁶</td>
<td>6.8 min</td>
<td>~1 h</td>
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<tr>
<td>Isolated*** 10,000</td>
<td>3.24x10⁴</td>
<td>0.3 min</td>
<td>&lt;10 min</td>
<td></td>
</tr>
</tbody>
</table>

* Flow rate: 2,000 events/s.
** Considering 9 samples (3 experimental groups × 3 replicas/group). Includes 45 s automated mixing and rinsing between samples on the MACSQuant instrument.
*** Isolation using CD8 (TIL), CD4 (TIL), or CD4/CD8 (TIL) MicroBeads, respectively.

For a short introduction to the procedure of TIL isolation, watch this video: miltenyibiotec.com/til-video

Figure 7: Isolation of tumor-associated fibroblasts from a mouse B16-F10 tumor (melanoma) using the Tumor-Associated Fibroblast Isolation Kit, mouse.

Figure 8: Isolation of TILs from mouse breast cancer tissue using CD45 (TIL) MicroBeads, mouse.

Figure 9: Isolation of TILs from mouse B16-F10 tumor models using mouse CD4 (TIL) MicroBeads, CD8 (TIL) MicroBeads, and CD4/CD8 (TIL) MicroBeads dramatically decreases time of analysis.

For cultivated mouse cells and human tumor cells, Nocodazole-stained (DAPI; blue), mouse cells are stained with anti-vimentin (red; mainly fibroblasts), and human tumor cells are stained with anti-EpCAM (green).
Tumor characterization with MACSQuant®
Flow Cytometers and MACS® Antibodies

Convenient flow cytometry analysis independent of the user’s experience:
• high-throughput flow cytometry analysis in a walk-away manner
• unique and innovative features, including rare cell enrichment
• precise absolute cell counting
• automated cell labeling

MACSQuant® VYB
Your benchmark for fluorescent protein flow cytometry

MACSQuant® Analyzer 16
Colorful new possibilities in automated flow cytometry

MACSQuant® X
Reliable high-throughput flow cytometry

MACSQuant® Analyzer 10
Your trusted partner in automated flow cytometry
Enhance the analysis of your tumor samples

MACS® Flow Cytometry

MACS® Flow Cytometry provides best-in-class solutions for all your tumor research needs. Optimize your results using REAfinity™ Recombinant Antibodies (fig. 10). Recombinantly engineered antibodies are the new standard for flow cytometry analysis. Their high purity and lot-to-lot consistency ensure reproducible results. Moreover, their mutated human IgG1 Fc region eliminates tedious and costly Fc receptor (FcR) blocking steps and allows for one universal isotype control, thus saving costs and providing maximal convenience.

Immunohistochemistry and Cell Enrichment and Detection Kits

MACS® Antibodies, as well as Cell Enrichment and Detection Kits, offer flexible options to enrich and analyze your target cells. Use MACS Antibodies for immunohistochemistry staining (fig. 11) or take advantage of the patented MACS Technology with Enrichment and Detection Kits: Target cells can be directly stained during separation, minimizing cell loss. Enriched cells can be used immediately, e.g., for flow cytometry.

Download our list of cancer-related antibodies here: miltenyibiotec.com/cancerabs

Figure 10: Splenocytes and tumor cells were isolated from BALB/c mice harboring H8N8 breast cancer tumors. Respective cells were subsequently labeled with Viobility™ 405/520 Fixable Dye, REA clones CD45-FITC, and CD3-APC, and either REA clone CD8α-PE or hybridoma clone CD8α-PE. Staining was performed in either presence or absence of FcR blocking reagent and viable CD45+ CD3– cells are shown.

Figure 11: Immunohistochemical localization of LGR5+ stem cells with MACS Antibodies. (A) Overview of human intestine crypts. (B) LGR5+ stem cells locate at the base of intestinal crypts in healthy tissue. (C) In adenoma, LGR5 staining shows an intensified and disorganized pattern of LGR5+ stem cells. [Courtesy of Michael K Dame, University of Michigan, Medical School]
Optimized culture conditions for pancreatic tumor cells:

- Efficient derivation and expansion of tumor cell cultures from pancreatic tumors
- Preserved parental tumor heterogeneity, tumor-initiating capacity, and genetic stability
- Improved in vitro models for pancreatic cancer research and drug screening
- Stable cell lines from primary tumor tissue

Culture is key with Pancreas TumorMACS™ Medium

Pancreas TumorMACS™ Medium
Serum-free medium composition optimized for the initiation and expansion of primary tumor cell cultures
Cultivating primary tumor cells

Stable primary cell lines from solid tumors

Generate stable primary cell cultures from solid tumor material following our standardized workflow: from tissue storage, tumor dissociation, cell isolation and flow cytometry, to the long-term cultivation of pancreatic tumor cells using Pancreas TumorMACS™ Medium (fig. 12).

Figure 12: Workflow overview on how to establish a stable cell line from primary pancreas tumor cells.

Tumors derived from established pancreatic cancer cell lines, such as PANC-1, fail to reflect the histological and functional features of primary human tumors (fig. 13), making the direct comparison to primary tissue impossible. In contrast, cell lines derived from primary tumors or patient-derived xenografts and propagated in Pancreas TumorMACS Medium retain their initial heterogeneity (fig. 14). This morphological and phenotypic heterogeneity as well as the preserved tumor-initiating capacity and genetic stability (fig. 15) lead to improved in vitro models.

Figure 13: PANC-1–derived xenografts show a homogeneous histology.

Figure 14: Patient-derived pancreatic tumor cells (PDAC) were injected into mice. Tumor tissue was extracted from the xenograft, cultured in Pancreas TumorMACS Medium over multiple passages, and reinjected into mice for the validation of tumorigenicity.

Figure 15: Gene expression profiles of early (passage 5) and late (passage 15) passages of the respective cell lines were analyzed. High correlation coefficients clearly demonstrate that the global gene expression profile is maintained upon long-term cultivation in Pancreas TumorMACS Medium (A). Likewise, the high level of correlation of cell line–derived xenotransplanted tumors (DT) to the initial patient tumors (PT) shows the preservation of genetic features (B).

For more information download our scientific poster here: miltenyibiotec.com/tumormacs
Exosome research solutions

Isolating exosomes the easy way:
• no ultracentrifugation
• marker-specific isolation for a precise analysis
• quick and easy protocol

Exosome Isolation Kit
Isolation of intact exosomes or extracellular vesicles (EVs) from cell culture supernatant, plasma, urine, or ascites

MACSPlex Exosome Kit
Detection of 37 exosomal surface epitopes plus two isotype controls
Magnetic exosome isolation and fast screening

Isolation of exosomes
Skip ultracentrifugation and isolate exosomes with MACS® Technology directly from cell culture supernatants, plasma, serum, urine, or ascites (fig. 16). The Exosome Isolation Kits target tetraspanin CD9, CD63, or CD81, or all three markers combined, allowing a precise isolation of your population of interest. Furthermore, using µ Columns and the µMACS Separator, MACS Technology allows the isolation of exosomes from sample volumes as little as 0.5–2 mL.

Fast screening of exosomes by flow cytometry
The MACS®Exo Assay Kit (fig. 17) enables an easy and fast screening of potential EV surface proteins (tab. 2):
- unique multiplex bead platform for protein profiling of EVs by flow cytometry
- saves precious sample material by screening 37 surface markers simultaneously
- analysis of EVs from cell culture supernatant or body fluids

Figure 16: Principle of magnetic isolation of extracellular vesicles (EVs) from cell culture supernatant or body fluids using an Exosome Isolation Kit.

Figure 17: Principle of the MACS® Exosense Kit. Isolated exosomes are incubated overnight with 39 differently labeled MACS® Exosense Capture Beads each coupled to a different antibody. Exosomes bound to the beads are detected with MACS® Exosense Detection Reagents by flow cytometry.

Figure 18: Surface marker profiles of EVs isolated from plasma by ultracentrifugation or immunomagnetic isolation using the Exosome Isolation Kits CD9, CD63, or CD81. EVs from 2 mL of plasma were isolated using one of the kits or by ultracentrifugation. Amounts were adjusted to a plasma volume of 2 mL, and exosomes were analyzed using the MACS® Exosense Kit. Data indicate median APC signal intensities of isolated EVs incubated with the 39 MACS® Exosense Capture Beads and stained with a cocktail of CD9-, CD63-, and CD81-APC antibodies. REA and mIgG1 indicate isotype control beads.

Traditionally, extracellular vesicles (EVs) are prepared by ultracentrifugation, which is time consuming and can lead to inconclusive results in protein profiling experiments. For example, EVs isolated by ultracentrifugation show only weak fluorescence signals when analyzed with the MACS®Exo Assay Kit (fig. 18). In contrast, optimal results are obtained after isolating EVs with Exosome Isolation Kits prior to analysis with the MACS®Exo Assay Kit (fig. 18).

Table 2: Overview of surface marker and control antibodies used for EV analysis by the MACS®Exo Assay Kit.

| Antibodies for EV analysis | CD90 | CD63.
|---------------------------|------|------
| Anti-HLA-ABC             | CD19 | CD63 | CD63
| Anti-CD19                | CD34 | CD39 | CD39
| Anti-HLA-DR, DP, DQ      | CD25 | CD81 | CD81
| Anti-MCSAP               | CD24 | CD24 | CD24
| Anti-ROR1                | CD25 | CD81 | CD81
| Anti-SSEA-4              | CD29 | CD36 | CD36
| CD1c                     | CD31 | CD90 | CD90
| CD2                       | CD40 | CD132.1/1
| CD3                      | CD40.1 | CD132.1
| CD4                      | CD42 | CD242 | CD242
| CD8                      | CD44 | CD244 | CD244
| CD9                      | CD45 | CD256 | CD256
| CD10                     | CD49 | Mouse IgG1 Control
| CD11c                    | CD56 | CD56 | CD56
| CD14                     | CD56 | CD56 | CD56

Figure 18: Surface marker profiles of EVs isolated from plasma by ultracentrifugation or immunomagnetic isolation using the Exosome Isolation Kits CD9, CD63, or CD81. EVs from 2 mL of plasma were isolated using one of the kits or by ultracentrifugation. Amounts were adjusted to a plasma volume of 2 mL, and exosomes were analyzed using the MACS®Exo Assay Kit. Data indicate median APC signal intensities of isolated EVs incubated with the 39 MACS® Exosense Capture Beads and stained with a cocktail of CD9-, CD63-, and CD81-APC antibodies. REA and mIgG1 indicate isotype control beads.

Figure 19: Fast screening of EVs using the MACS®Exo Assay Kit.
Sample preparation

<table>
<thead>
<tr>
<th>Product</th>
<th>Order no.</th>
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<tbody>
<tr>
<td>Tumor Dissociation Kit, human*</td>
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<tr>
<td>Tumor Dissociation Kit, mouse</td>
<td>130-096-760</td>
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<tr>
<td>Brain Tumor Dissociation Kit (P)**</td>
<td>130-095-942</td>
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<tr>
<td>FFPE Tissue Dissociation Kit</td>
<td>130-118-052</td>
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<tr>
<td>MACS® Tissue Storage Solution</td>
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</tbody>
</table>

* For human or xenograft tumors.
** For the analysis of immune cells in brain tumors, use the Tumor Dissociation Kit, human or mouse.

Cell analysis

For a complete list of antigens and conjugates visit [www.miltenyibiotec.com/antibodies](http://www.miltenyibiotec.com/antibodies)

To find out about the advantages of using REAfinity™ Recombinant Antibodies (REA Clones) for cell analysis, visit [www.miltenyibiotec.com/reafinity](http://www.miltenyibiotec.com/reafinity)

Cell culture

<table>
<thead>
<tr>
<th>Product</th>
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<td>Pancreas TumorMACS™ Medium</td>
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Exosome research

<table>
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<td>Exosome Isolation Kit Pan, human</td>
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<td>Exosome Isolation Kit CD9, human</td>
<td>130-110-913</td>
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<td>Exosome Isolation Kit CD63, human</td>
<td>130-110-918</td>
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<td>Exosome Isolation Kit CD81, human</td>
<td>130-110-914</td>
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<td>MACSPlex Exosome Kit, human</td>
<td>130-108-813</td>
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Mouse

| Exosome Isolation Kit Pan, mouse             | 130-117-039|
| Exosome Isolation Kit CD9, mouse             | 130-117-042|
| Exosome Isolation Kit CD63, mouse            | 130-117-041|
| Exosome Isolation Kit CD81, mouse            | 130-117-040|

Cell separation

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<td>Mouse Cell Depletion Kit</td>
<td>130-104-694</td>
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<td>Carcinoma Cell Enrichment Kit, human</td>
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<td>CD326 (EpCAM) MicroBeads, human</td>
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<td>CD326 (EpCAM) Tumor Cell Enrichment and Detection Kit, human</td>
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<td>StraightFrom® Whole Blood CD326 (EpCAM) MicroBeads, human</td>
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<td>CD133 MicroBead Kit – Tumor Tissue, human</td>
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<td>StraightFrom Whole Blood CD138 MicroBeads, human</td>
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<td>B-CLL Cell Isolation Kit, human</td>
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<td>MACSexpress® Whole Blood B-CLL Cell Isolation Kit, human</td>
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<td>EPC Enrichment and Enumeration Kit, human</td>
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<td>cEUC Enrichment &amp; Enumeration Kit, human</td>
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<td>Anti-LGR5 MicroBeads, human</td>
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<td>CD31 MicroBead Kit, human</td>
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Mouse

| Exosome Isolation Kit Pan, mouse             | 130-110-187|
| Exosome Isolation Kit CD9, mouse             | 130-116-474|
| CD326 (EpCAM) MicroBeads, mouse              | 130-105-958|
| CD45 (TIL) MicroBeads, mouse                 | 130-110-618|
| CD4 (TIL) MicroBeads, mouse                  | 130-116-475|
| CD8 (TIL) MicroBeads, mouse                  | 130-116-478|
| CD4/CD8 (TIL) MicroBeads, mouse              | 130-116-480|
| CD31 MicroBeads, mouse                       | 130-097-418|

For more options of MicroBeads for direct selection of cells from tumors please visit [www.miltenyibiotec.com](http://www.miltenyibiotec.com) and look for your marker of interest.

● Indicated for cells from solid tumors

● Indicated for cells from liquid tumors
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