MACS
Miltenyi Biotec

MSC research

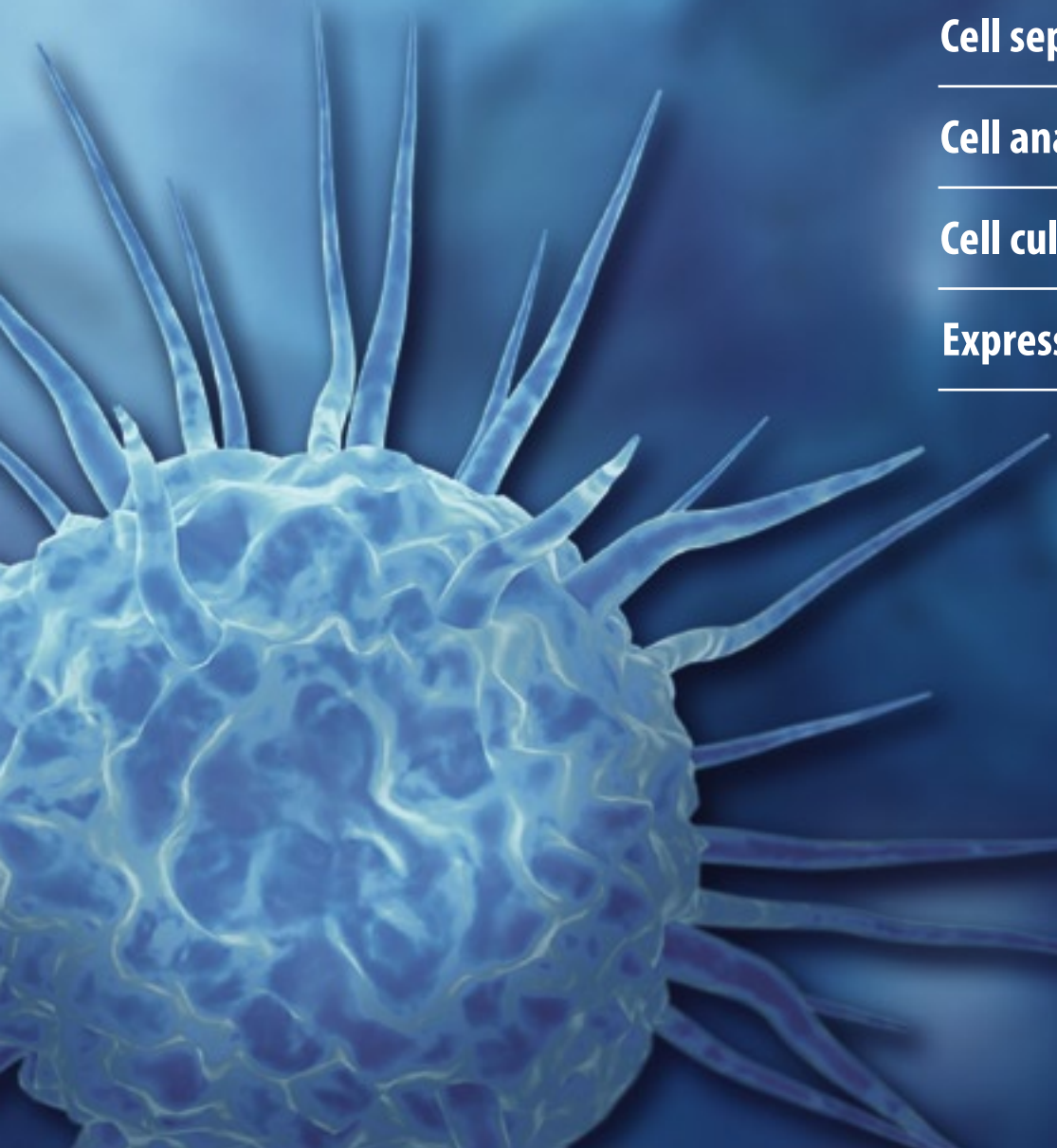
Sample preparation

Cell separation

Cell analysis

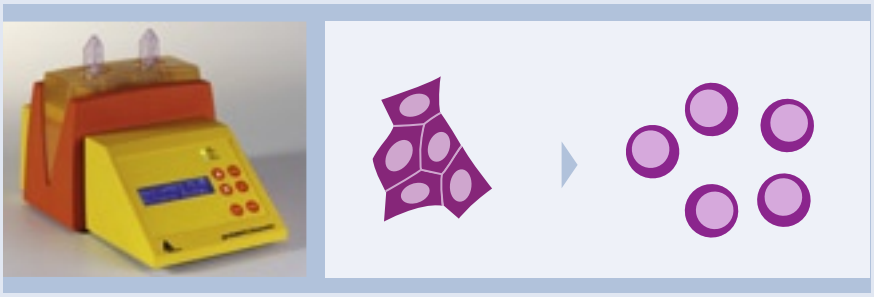
Cell culture

Expression profiling



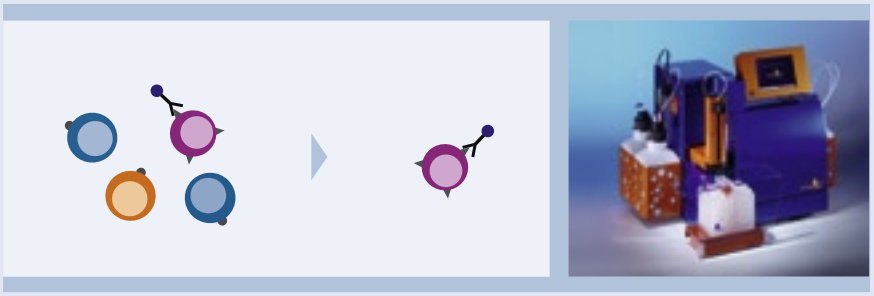
MACS® Technology —the complete solution

Since its introduction in 1989, MACS® Technology has become the gold standard for cell separation. Nowadays, Miltenyi Biotec stands for more than cell separation, offering more than 1000 innovative research products for biomedical research and life sciences. The MACS Research Product portfolio includes instruments and reagents for sample preparation, cell separation, cell analysis, cell culture, and molecular biology.



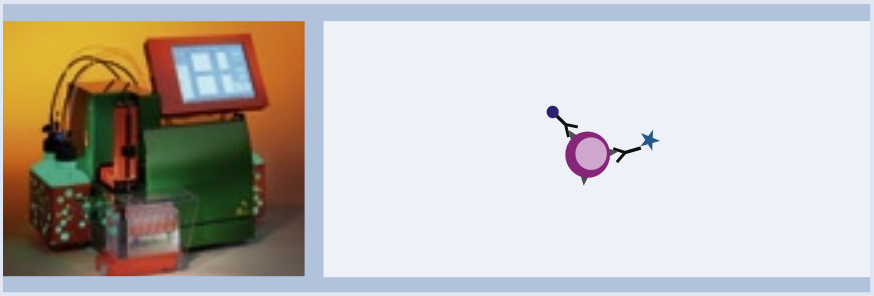
MACS Sample Preparation

The quality of an experiment strictly depends on the quality of the sample preparation.
Miltenyi Biotec offers innovative instruments and reagents for fast and gentle preparation of viable single cells from solid tissues as well as cultured cells.



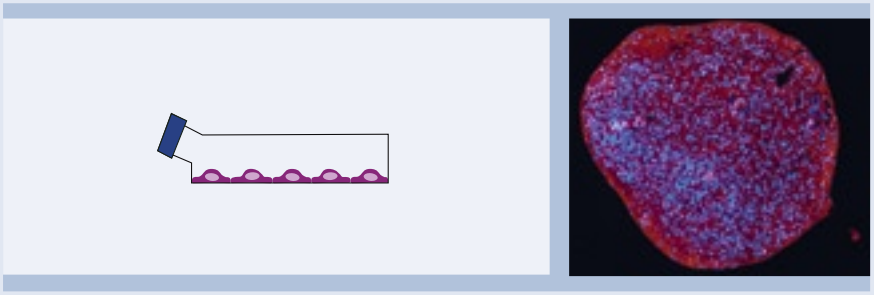
MACS Cell Separation

A large panel of MACS MicroBeads and MicroBead Kits is available for the isolation of virtually any cell type, including MSCs from human and mouse. The cells can be separated manually or in an automated fashion. The new autoMACS™ Pro Separator has been designed for automated walk-away cell sorting of multiple samples.



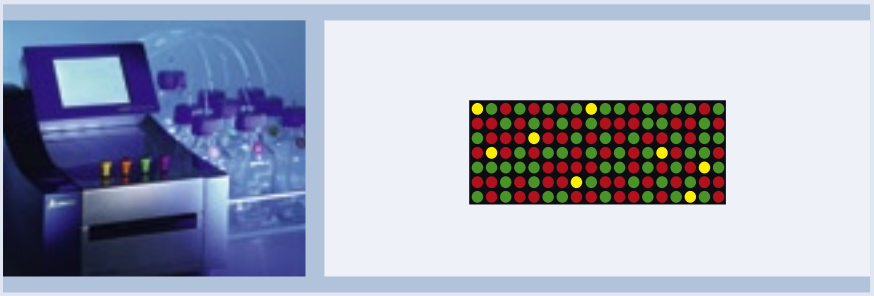
MACS Cell Analysis

Miltenyi Biotec provides a large panel of monoclonal antibodies and kits for fluorescence microscopy and flow cytometry. The innovative MACSQuant™ Analyzer is an extremely compact, easy-to-use, multicolor benchtop cell analyzer. The instrument is fully automated and enables absolute cell count.



MACS Cell Culture

The product portfolio for cell culture includes specially developed media for the expansion, differentiation, and enumeration of MSCs. Recombinant cytokines and growth factors are available for expansion and differentiation of MSCs into a wide variety of cell types.



MACSmolecular

Miltenyi Biotec provides products for analytical protein isolation and detection, mRNA purification and amplification, cDNA synthesis and labeling, microRNA analysis, as well as microarray technologies and instrumentation. The portfolio includes genomics services, such as gene expression and microRNA analysis microarrays, array-CGH, and bioinformatics.

Multipotent mesenchymal stromal cells

Multipotent mesenchymal stromal cells (MSCs) are fibroblast-like plastic-adherent cells that can be isolated from a variety of tissues, such as bone marrow, periosteum, trabecular bone, adipose tissue, synovium, skeletal muscle, dental pulp, and other tissues. In adult bone marrow, MSCs are present at a very low frequency, i.e., less than one MSC per 10^4–10^5 bone marrow mononuclear cells and about one MSC in 10^2–10^3 cells from lipoaspirate.[1] MSCs are defined by their potential to differentiate into connective tissue cells, such as adipocytes, chondrocytes, and osteoblasts.

MSCs are known under various names. Therefore, there are efforts to establish a standard name for this cell type: the International Society for Cellular Therapy (ISCT) introduced the term multipotent mesenchymal stromal cells.[2]

MSC research may provide the basis for a variety of potential clinical applications

Tissue regeneration

MSCs have a differentiation potential beyond mesodermal fate and are able to differentiate into a broad variety of nonhematopoietic (NH) tissues *in vitro*, e.g., liver or neural tissue. In animal studies, MSCs were able to engraft in multiple tissues after intraveneous infusion[3]. MSCs show site-specific differentiation, raising the hope that MSCs could be used for the regeneration of damaged and diseased tissue in humans, e.g., for neural or orthopedic[4] applications.

Immunomodulation

MSCs are immunoprivileged and modulate T and B cell proliferation and differentiation, dendritic cell maturation, and natural killer activity *in vitro*[5–10]. They have been successfully used for the treatment of graft-versus-host disease (GVHD) in clinical settings[11,12].
Furthermore, the use of MSCs for immunosuppressive therapy in human patients suffering from diabetes and/or other tissue-destructive autoimmune diseases is of great interest and the underlying mechanism is currently under investigation.

Expansion of HSCs *in vitro*

Bone marrow MSCs contribute to the bone marrow reticular niche and play an important role in the bone marrow microenvironment. They provide growth factors and matrix proteins, which play a role in the regulation of cell-to-cell interactions during hematopoiesis. MSCs provide factors required for the maintenance as well as the development of hematopoietic stem cells (HSCs): MSCs maintain primitive hematopoietic progenitor cells *in vitro*[13], improve expansion of cord blood HSCs[14] and the generation of red blood cells *in vitro*[15].

Improved engraftment of HSCs

MSCs are a crucial component of the the stem cell niche and form a supporting structure for the homing of HSCs. Therefore, MSCs have been considered as a possible tool to ameliorate HSC engraftment in an allogenic transplantation setting. Indeed, MSCs promote engraftment of cord blood–[16] and bone marrow–derived[17] $CD34^+$ cells in NOD/SCID mice when co-administered; the presence of human MSCs in murine bone marrow results in an increase in functionally and phenotypically primitive human hematopoietic cells under these experimental conditions. Human MSC-derived cells that reconstitute the hematopoietic microenvironment appear to contribute to the maintenance of hematopoiesis by interacting with primitive human hematopoietic cells.[18]
In a clinical setting, it was shown that cotransplantation of *ex vivo* expanded MSCs accelerates lymphocyte recovery and may reduce the risk of graft failure in haploidentical hematopoietic stem cell transplantation.[19]

Delivery vehicle, e.g., for potential treatment of cancer or genetic disorders

Tumors are composed of malignant tumor cells and non-malignant cells, such as blood vessels and stromal fibroblasts, which provide the structural support. Recent evidence suggests that MSCs selectively home to tumors, such as ovarian carcinomas or even gliomas, where they contribute to the formation of tumor-associated stroma. Accordingly, MSCs have been used as an effective cellular vehicle for the targeted delivery and local production of anticancer agents, such as therapeutic proteins, at cancer sites in animal models.[20–22]
MSCs have also been explored as vehicles for gene therapy, e.g., for hemophilia B, based on their potential to engraft and deliver certain secreted proteins over a long period of time.[23,24]

To investigate the potential of MSCs in clinical applications, it is crucial to standardize their isolation and culture methods. Miltenyi Biotec has developed the most comprehensive portfolio for the efficient isolation and expansion of stem cells.

References

1. Kern, S. *et al.* (2006) Stem Cells 24: 1294–1301.
2. Horwitz, E. M. *et al.* (2005) Cytotherapy 7: 393–395.
3. Kan, E. *et al.* (2005) Curr. Drug Targets 6: 31–41.
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6. Nauta, A. J. *et al.* (2006) J. Immunol. 177: 2080–2087.
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11. Le Blanc, K. *et al.* (2004) Lancet 363: 1439–1441.
12. Nauta, A. and Fibbe, W. E. (2007) Blood 110: 3499–3506.
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23. Hurwitz, D. R. *et al.* (1997) Hum. Gene Ther. 8: 137–156.
24. Cherington, V. *et al.* (1998) Hum. Gene Ther. 9: 1397–1407.

Sample preparation

Preparation of cell suspensions for the isolation of MSCs

MSCs can be isolated from a broad variety of tissues.[1,2] The preparation of suspensions of viable single cells from tissues is a critical step towards the subsequent isolation of MSCs. Miltenyi Biotec offers the new gentleMACS™ Dissociator for the gentle and efficient dissociation of tissues. Pre-Separation Filters and the Dead Cell Removal Kit allow an optimal preparation of cell suspensions for subsequent separation.

References

1. da Silva Meirelles, L. *et al.* (2006) J. Cell Sci. 119: 2204–2213.
2. Covas, D. *et al.* (2008) Exp. Hematol. 36: 642–654.

gentleMACS™ Dissociator

The gentleMACS Dissociator is a benchtop instrument for the automated dissociation of tissues. Two types of unique gentleMACS Tubes enable the time-saving and easy dissociation of tissues to single-cell suspensions or homogenization of tissues to lysates in a closed system. The range of protocols available for the gentle and efficient dissociation of tissues will be continuously expanded. Protocols are optimized to yield single-cell suspensions with a high viability rate.
The gentleMACS Dissociator can further be used for the homogenization of tissues or cells to extract biomolecules for molecular biology experiments, such as the isolation of total RNA or mRNA.

Features at a glance

- Time-saving automated tissue dissociation or homogenization
- Standardized procedure
- Reliable and reproducible results
- High level of user safety
- Sterile sample handling

Pre-Separation Filters

These 30 µm nylon mesh filters are used to filter cell suspensions in order to remove cell clumps prior to applications where single-cell suspensions are required, for example, cell separation. This can improve the separation performance, especially when isolating very rare cells.

Dead Cell Removal Kit

The Dead Cell Removal Kit is a fast and straightforward way of eliminating dead cells from cell cultures or tissue preparations. The Dead Cell Removal Kit contains ready-to-use MicroBeads and Binding Buffer for the magnetic labeling of cell debris, dead cells, and dying cells. The magnetically labeled material is removed by magnetic separation and pure, viable cells are obtained within 25 minutes.

Lipoaspirate— a valuable source of MSCs

Lipoaspirate, an otherwise disposable byproduct of cosmetic surgery, contains a putative population of stem cells, termed adipose-derived stem cells (ADSCs). These cells resemble MSCs from bone marrow, including their multilineage differentiation capacity.[1] The use of lipoaspirate as a source for stem cells with multipotent differentiation potential offers a far less invasive procedure for cell sampling than the aspiration of bone marrow. Moreover, lipoaspirate can be obtained in large quantities.
These cells show high frequencies of colony-forming units[2,3] and may have a pluripotent ability to differentiate, e.g., into cells of a neuronal phenotype[1] in addition to the mesenchymal lineages.
Miltenyi Biotec offers a special protocol for the isolation of MSCs from lipoaspirate. The complete protocol is available at www.miltenyibiotec.com.

References

1. Zuk, P. A. *et al.* (2002) Mol. Biol. Cell 13: 4279–4295.
2. Kern, S. *et al.* (2006) Stem Cells 24: 1294–1301.

Product	Components or capacity	Order no.
gentleMACS™ Starting Kit	gentleMACS™ Dissociator C Tubes, 25 pieces M Tubes , 25 pieces Power cord User manual gentleMACS Protocols	130-093-235
C Tubes	C Tubes, 25 pieces, single-packed	130-093-237
M Tubes	M Tubes, 25 pieces, single-packed M Tubes, 50 pieces per bag	130-093-236 130-093-458
Pre-Separation Filters	50 filters	130-041-407
Dead Cell Removal Kit	For 10^9 total cells	130-090-101

MACS® Technology for stem cell separation

The preferred choice for stem cell separation

MACS® Cell Separation

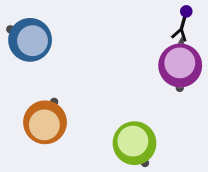
MACS® Technology is a robust tool for the isolation of defined cell populations and subpopulations to high purity.
MACS Technology for cell separation is based on the use of MACS MicroBeads—antibody-coupled superparamagnetic particles—as well as MACS Separators and MACS Columns.
The cells are labeled with MACS MicroBeads to be magnetically retained within the MACS Column, when it is placed in the magnetic field of the MACS Separator. Target cells can be isolated by positive selection or by depletion of unwanted cells in order to obtain untouched cells. These two separation strategies can be combined to provide greater flexibility for sequential sorting of complex subpopulations of cells. Alternatively, MACS MultiSort technology enables the sequential positive selection of cells for the isolation of defined subpopulations.
The MACS Cell Separation process is fast and efficient. After separation, the cells can be directly used in downstream applications, such as functional assays or cell expansion.

MACS MicroBeads

- Highly specific monoclonal antibody conjugates
- Small (50 nm), virus-sized nanoparticles
- Non-toxic, biodegradable
- Colloidal, for ease of handling and short incubation times

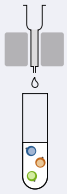
MACS Columns and MACS Separators

- High-gradient magnetic field
- Optimal recovery and high purity with MACS Columns
- Gentle to cells
- Automated cell separation with autoMACS™ Pro Separator



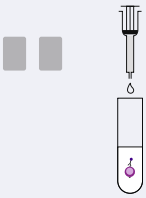
Magnetic labeling

Cells of interest are labeled with MACS® MicroBeads in a short incubation step.



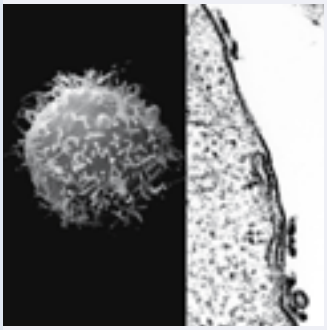
Magnetic separation

Labeled and unlabeled cells are separated on a MACS Column placed in the magnetic field of a MACS Separator. The flow-through can be collected as the non-magnetic, unlabeled fraction.



Elution of the labeled cell fraction

The separation column is removed from the magnetic field and the retained cells are flushed out. Both the labeled and unlabeled fractions can be recovered and used for downstream applications.



Due to the extremely small size of MACS MicroBeads, the separated cells can be directly analyzed by flow cytometry or used in functional assays. MACS MicroBeads have no effects on the scatter properties of cells in flow cytometry and are biodegradable.

MidiMACS™ Separator

autoMACS™ Pro Separator

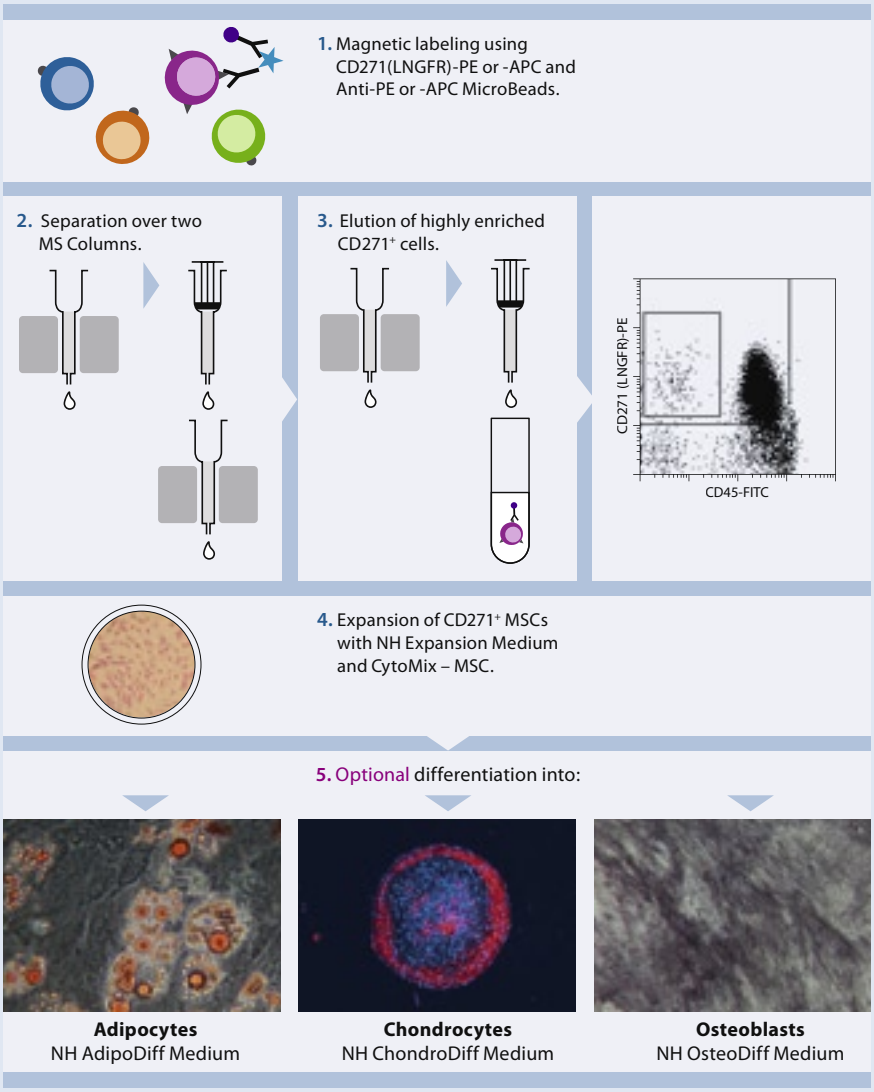
MSC isolation and analysis

To investigate the potential of MSCs for future clinical applications and to explore possible therapies, it is important to understand and control the processes and factors that mediate immunomodulation and the capacity of MSCs to differentiate into functional nonhematopoietic cell types. Homogenous MSC populations are required to obtain reliable results. Thus, the establishment of efficient and reproducible procedures for the isolation of MSCs is essential. Miltenyi Biotec is a long-standing provider of high-quality kits and reagents for the separation of a wide range of cell types from differing species. Numerous tools are available for the efficient isolation and analysis of MSCs according to various markers.

Isolation and analysis of human MSCs

CD271—a marker for the isolation of MSCs from bone marrow and lipoaspirate

CD271, also known as low-affinity nerve growth factor receptor (LNGFR) of p75 or p75NTR, belongs to the low-affinity neurotrophin receptor and the tumor necrosis factor receptor superfamily. It is a well-known marker for the isolation of MSCs directly from bone marrow aspirate.[1–4] After separation, colony-forming unit fibroblast (CFU-F) activity was found only in the CD271$^+$ cell fraction. These cells have a 10- to 100-fold higher proliferative capacity compared to MSCs isolated by plastic adherence.[4] Furthermore, CD271 is a specific marker for MSCs from lipoaspirate.[5] CD271 is also expressed on MSCs from synovial fluid.[6]



Isolation, expansion, and further differentiation of CD271$^+$ cells using the MSC Research ToolBox – CD271 (LNGFR) and NH Differentiation Media.

References

1. Jones, E. A. *et al.* (2004) (Abstract) 4th Annual Conference on Mesenchymal and Nonhematopoietic stem cells.
2. Jones, E. A. *et al.* (2006) Cytometry B Clin. Cytom. 70: 391–399.
3. Jones, E. A. *et al.* (2007) MACS&more 11-1: 22–25. Reprinted on pp. 8–11 of this publication.
4. Quirici, N. *et al.* (2002) Exp. Hematol. 30: 783–791.
5. Meyerrose *et al.* (2007) Stem Cells 25: 220–227.
6. Jones, E. A. *et al.* (2004) Arthritis Rheum. 50: 817–827.

The CD271 (LNGFR) MicroBead Kits (PE) or (APC) are indirect labeling systems for the immunomagnetic isolation of CD271 (LNGFR)$^+$ cells. The kits include all necessary reagents for fluorescent labeling, i.e., CD271 (LNGFR)-PE or -APC, and indirect magnetic labeling, i.e., Anti-PE or -APC MicroBeads.

The MSC Research Tool Box – CD271 (PE) or (APC) have been developed for the standardized and optimized enrichment and expansion of MSCs from bone marrow aspirates. The tool boxes contain all the reagents for fluorescent labeling, i.e., CD271 (LNGFR)-PE or -APC, and indirect magnetic labeling, i.e., Anti-PE or -APC MicroBeads. Furthermore, it includes NH Expansion Medium, a specialized medium for the expansion of MSCs, and CytoMix – MSC, a cytokine cocktail designed specifically for the optimal and reproducible expansion of pre-selected MSCs. A special protocol for the isolation of MSCs from lipoaspirate based on the expression of CD271 is available at www.miltenyibiotec.com. The CD271 antibody cross-reacts with non-human primate, bovine, swine, and dog.

Product	Order no.
MSC Research Tool Box – CD271 (PE)	130-092-867
MSC Research Tool Box – CD271 (APC)	130-092-291
CD271 MicroBead Kit (PE)	130-092-819
CD271 MicroBead Kit (APC)	130-092-283
CD271 (LNGFR), -PE, -APC, -Biotin	130-091-885, 130-091-884, 130-091-883

MSC isolation and analysis

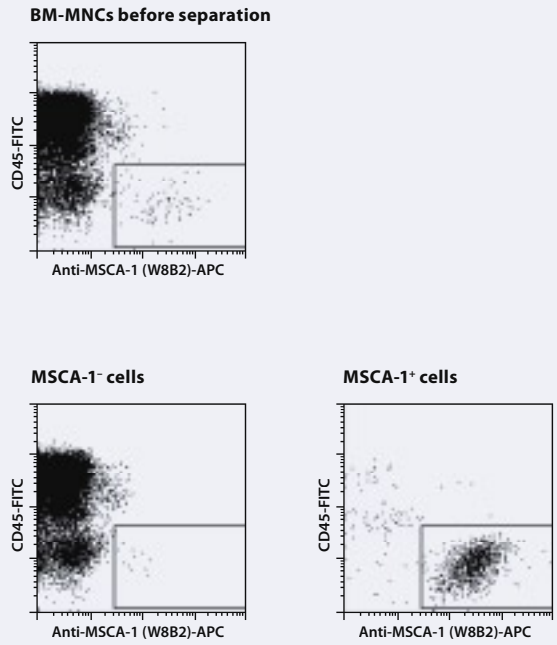
MSCA-1 (W8B2)—a specific marker for the isolation of MSCs from bone marrow

The mesenchymal stromal cell antigen–1 (MSCA-1), identified by the clone W8B2, is restricted to MSCs in the CD271^{bright} population in bone marrow.[1] These CD271^{bright}CD45^{dim} MSCs have a much higher clonogenic capacity compared to the CD271^{+}CD45^{+} fraction in bone marrow.[1] Therefore, MSCA-1 is a suitable marker for the identification of MSCs with a high proliferative potential.

Reference

1. Bühring, H. J. *et al.* (2007) Ann. NY Acad. Sci. 1106: 262–271.

The Anti-MSCA-1 (W8B2) MicroBead Kit has been developed for the standardized and optimized enrichment of human MSCs from bone marrow aspirates.
The MSC Research Tool Box – MSCA-1 (W8B2) is designed for the standardized and optimized enrichment and expansion of human MSCs from bone marrow aspirates. The tool box contains all necessary reagents for the magnetic isolation of MSCA-1^{+} cells. Furthermore, the MSC Research Tool Box MSCA-1 (W8B2) contains NH Expansion Medium, a specialized medium for the expansion of MSCs, and CytoMix – MSC, a cytokine cocktail designed specifically for the optimal and reproducible expansion of pre-selected MSCs.
The Anti-MSCA-1 (W8B2) antibodies can be used to identify and enumerate MSCs with high proliferative capacity from bone marrow or other tissues, such as lipoaspirate, by flow cytometry or fluorescence microscopy.



MSCA-1 (W8B2)$^{+}$ cells were isolated from human bone marrow mononuclear cells (BM-MNCs) using the MSC Research Tool Box – MSCA-1 (W8B2). Cells were stained with Anti-MSCA-1 (W8B2)-APC and CD45-FITC.

Product	Order no.
MSC Research Tool Box – MSCA-1 (W8B2)	130-093-572
Anti- MSCA-1 (W8B2) MicroBead Kit	130-093-583
Anti- MSCA-1 (W8B2), -PE, -APC, -Biotin, pure	130-093-587, 130-093-589, 130-093-593, 130-093-595

Anti-fibroblast antigen

This antigen is expressed on MSCs and putative mesenchymal progenitor cells in bone marrow[1–3], and on fibroblasts. Anti-Fibroblast MicroBeads have been developed for the positive selection or depletion of human fibroblasts using a fibroblast-specific antigen.[4] However, applications also include the isolation of MSCs from bone marrow.[1–3]

References

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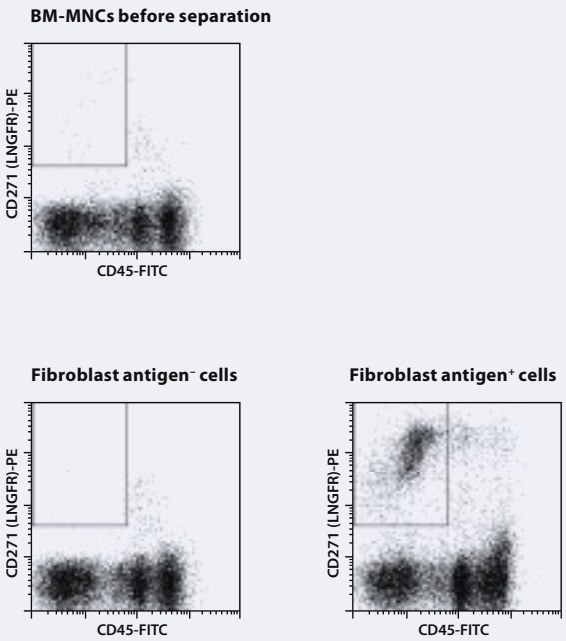
2. Jones, E. A. *et al.* (2006) Cytometry B: Clin. Cytom. 70: 391–399.
3. Jones, E. A. *et al.* (2007) MACS&more 11-1: 22–25. Reprinted on pp. 8–11 of this publication.
4. Fearns, C. and Dowdle, E. B. (1992) Int. J. Cancer 50: 621–627.

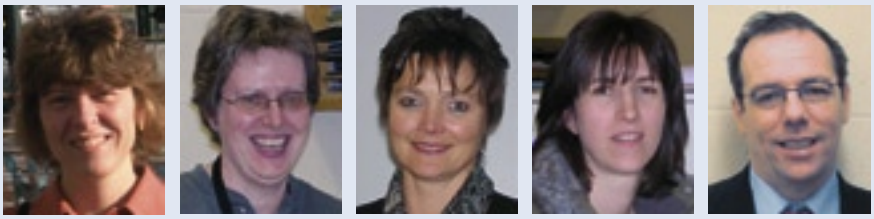
Product	Order no.
Anti-Fibroblast MicroBeads, human	130-050-601



Fibroblast antigen–positive cells were isolated from bone marrow mononuclear cells (BM-MNCs) using the Anti-Fibroblast MicroBeads. Cells were stained with CD271 (LNGFR)-PE and CD45-FITC. (Courtesy of Dr. Elena Jones, Leeds, UK.)

Purification of proliferative and multipotential marrow stromal cells (MSCs) from bone marrow aspirate by selection for CD271 (LNGFR) expression

Excerpt from MACS&more (2008) 11-1: 22–25.



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[3]The Division of Experimental Haematology, Leeds Institute for Molecular Medicine, University of Leeds, UK

Introduction

Stromal cells provide a niche and a cytokine support for hematopoietic cell maturation in the bone marrow (BM). At the same time, they form a pool of precursors for BM adipocytes and osteoblasts[1] which may include the most primitive, multipotential stromal cells (MSCs). BM MSCs can be isolated based on their expression of several surface antigens including STRO-1, CD146, fibroblast antigen (D7-FIB), CD73, CD105, and CD271 (LNGFR).[2–6] In addition, the CD45^{low} phenotype of freshly isolated BM MSCs has been demonstrated in several independent studies.[3,5,7] In this study we evaluate several MSC surface markers for their selectivity in detecting the CD45^{low} BM MSC population following pre-enrichment by separation with Anti-Fibroblast MicroBeads. Having established that CD271 (LNGFR) provided the best discriminative value, we compared MSC isolation methods based on selection for CD271 (LNGFR) expression to the separation with Anti-Fibroblast MicroBeads in terms of their (1) ability to generate proliferative and multipotential MSC cultures and (2) efficiency in isolating fresh BM MSCs.

Materials and methods

BM aspirates were obtained from the iliac crest of 14 healthy donors. Standard MSC cultures were established from plastic-adherent BM cell fractions.[8] For positive cell selection using MACS® Technology, 5×10^7 BM mononuclear cells were labeled with either Anti-Fibroblast MicroBeads or MACSelect LNGFR MicroBeads. MSC expansion, tri-lineage differentiation, flow cytometry, and cell sorting were performed as described previously.[3,9] All antibodies were purchased from Pharmingen, with the exception of CD105-PE (Serotec) and CD45-FITC (DAKO). Cell proliferation rate was assessed in an XTT-based colorimetric assay after MSCs expansion in either MACS® NH Expansion Medium or DMEM supplemented with 10% FCS. Differentiation of MSCs to cells of adipocyte, chondrocyte, and osteoblast lineages was performed using MACS® NH AdipoDiff Medium, NH ChondroDiff Medium, and NH OsteoDiff Medium.

Results

Comparative analysis of MSC marker selectivity

A good marker for fresh, uncultivated BM MSCs should exhibit a high level of expression on rare MSCs and minimal expression on cells of the predominant hematopoietic lineage cells (HLCs). To compare the selectivity of different markers, MSCs were first selected by using Anti-Fibroblast MicroBeads and then analyzed for the particular marker expression levels on gated CD45^{low} MSCs or CD45^{high} HLCs. Figure 1 demonstrates that CD271 (LNGFR) shows a very high, homogenous level of expression on MSCs as well as minimal labeling of HLCs. CD73 and CD13 were strongly expressed on MSCs, but showed increased staining of HLCs. CD105 and CD146 displayed more heterogeneous and weaker expression on MSCs, and an intermediate expression on HLCs. Based on these data, we concluded that CD271 (LNGFR) could be an excellent marker for the high-purity isolation of MSCs from human BM.

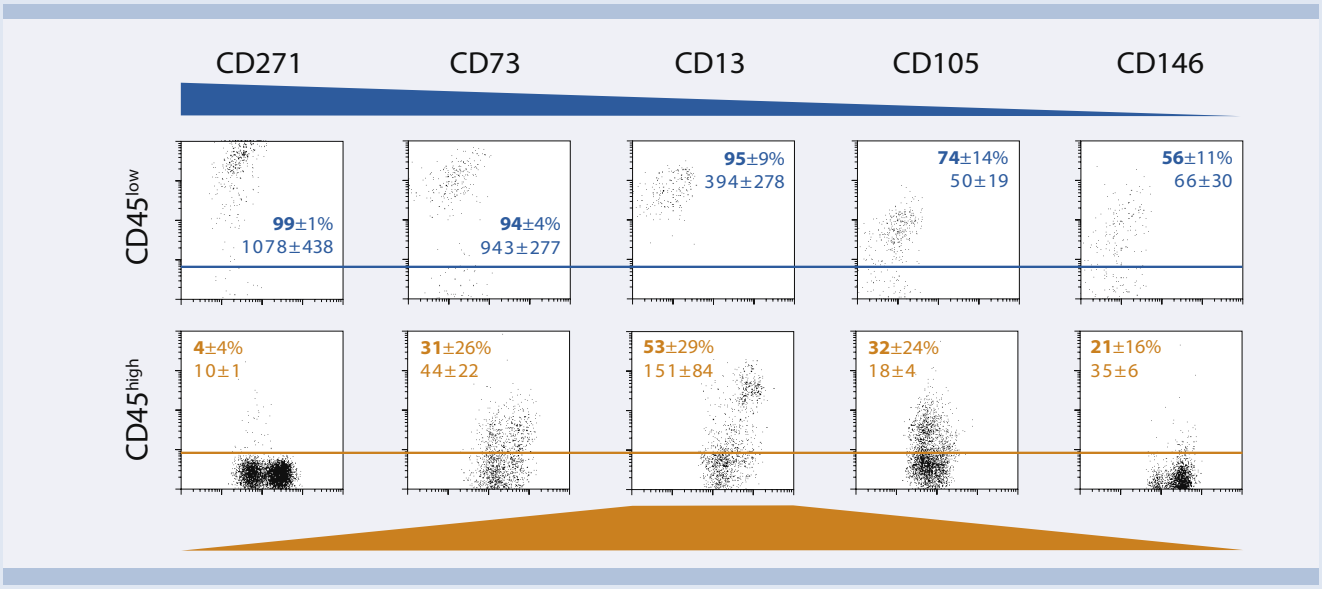

Figure 1 Comparative analysis of MSC marker selectivity. MSCs were pre-enriched with Anti-Fibroblast MicroBeads; marker expression levels were analyzed on gated CD45^{low} MSCs (top panel) and CD45^{high} hematopoietic lineage cells (HLCs) (bottom panel). Percentage and mean fluorescence intensity of marker-positive cells for MSCs (shown in blue) and HLCs (shown in orange) represent a mean ± SD for a minimum of 5 donors.

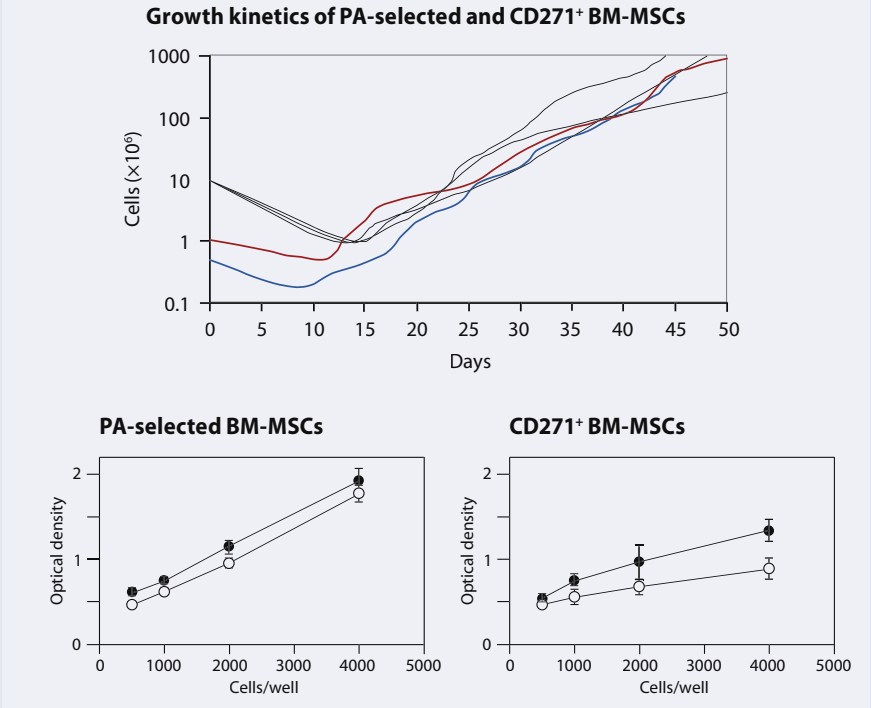

Figure 2 Growth characteristics of cells isolated using MACS® Technology vs. plastic adhesion-enriched cells (PA). (A) Expansion kinetics of BM cells isolated for CD271 (LNGFR) expression (blue line) compared with cells isolated with Anti-Fibroblast MicroBeads (red line) and three control cultures expanded from plastic-adherent BM cells (black lines). **(B)** Cell yields (measured as optical densities in the duration of a four-day XTT proliferation assay) in control cultures derived from BM plastic-adherent fraction (left panel) and cultures from CD271 (LNGFR)–selected cells (right panel). MACS® NH Expansion Medium (dark circles) produced higher cell yields than conventional DMEM/10% FCS (empty circles).

Growth characteristics of cells selected for CD271 (LNGFR) or fibroblast antigen expression

Cells isolated by MACS® Technology displayed similar growth characteristics to control cultures that had been expanded from plastic-adherent BM cell fractions, however they entered an exponential growth phase several days earlier (fig. 2A). Similarly to controls, cultures derived from the cells, isolated with MACSelect LNGFR MicroBeads, were formed by spindle-shaped cells and showed surface marker profiles consistent with cultured BM MSCs[3,8] (table 1). Cultures grown in MACS® NH Expansion Medium proliferated faster than cultures grown in conventional DMEM/10% FCS, regardless of whether or not the cell isolation step was used before culturing (fig. 2B).

Differentiation potentials of cultures derived from isolated cells

Similarly to cultures, isolated by using Anti-Fibroblast MicroBeads[3], MSC cultures established from cells selected for CD271 (LNGFR) expression retained full tri-lineage differentiation potential (fig. 3). In quantitative *in vitro* differentiation assays[9], levels of differentiation (measured by production of adipocytes, Ca^{2+}, or sulfated glycosaminoglycans) in cultures maintained in MACS® NH AdipoDiff,

	CD45	CD34	CD133	CD105	CD73	CD13	CD106	CD146	CD166	FA*
Standard MSC culture	0	0	0	98	99	98	52	81	99	95
Fibroblast antigen–selected cells	0	0	0	97	98	98	78	63	98	81
CD271 (LNGFR)–selected cells	0	0	0	91	100	99	37	69	100	82

All values are % positive cells * Fibroblast antigen

Table 1 Cell surface marker profiles of growing MSC cultures.

NH OsteoDiff, and NH ChondroDiff Media were 2-fold to 5-fold higher than those in cultures grown in respective home-made differentiation media.

Purification of adherent stromal cells in fractions isolated with MACS® Technology

One day after magnetic selection for either CD271 (LNGFR) or fibroblast antigen expression, the same morphologically distinct adherent cell type could be observed in the positive fractions (fig. 4A, left panel), but not in the negative fractions (right panel). These cells had a stromal (spindle-shape or stellate) morphology and cytoplasmic projections. Seeded on grid coverslips, the fate of individual adherent cells could be followed for several days[3], and their exponential growth was observed from as early as day three of culture (fig. 4B), whereas small round cells did not amplify.

As the cells, selected Anti-Fibroblast MicroBeads, cell fractions isolated for CD271 (LNGFR) expression contained a population of $CD45^{low}CD73^{+}$ cells (fig. 4C). This population was present at a much lower proportion before MACS® Separation and absent in the negative cell fraction. Cells sorted for the $CD45^{low}CD73^{+}$ phenotype yielded a pure population of stromal cells (fig. 4D) and contamination with small HLCs was no longer observed.

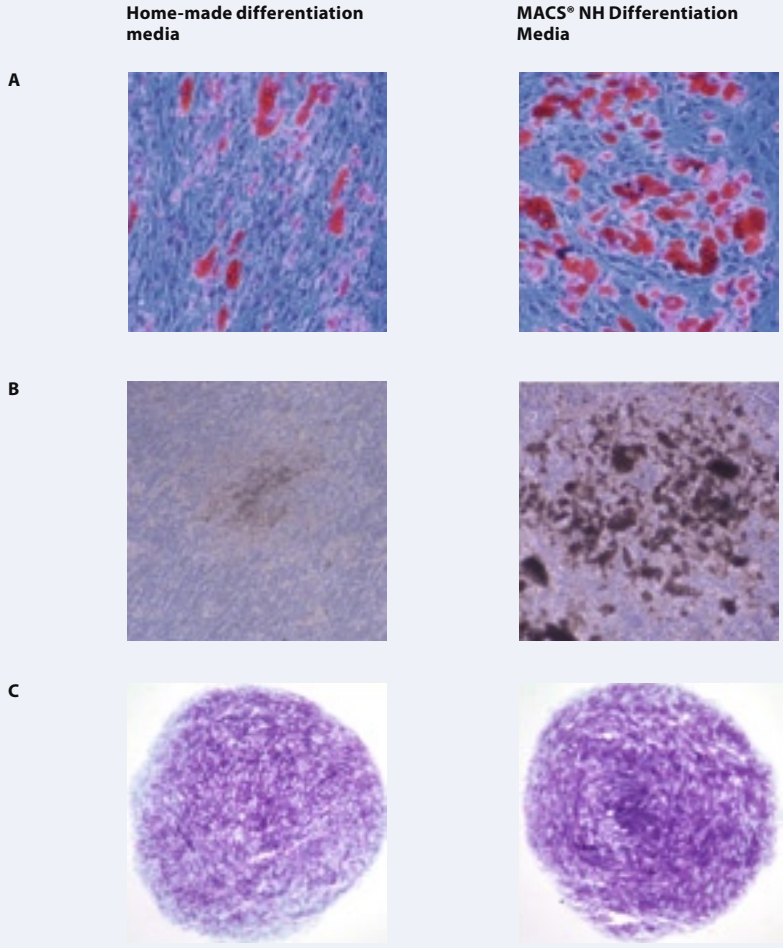

Figure 3 The effect of differentiation media on tri-lineage differentiation potential of CD271 (LNGFR)–isolated cells.
The same "passage 2" cell culture, derived from CD271 (LNGFR)–isolated cells, was used in all differentiation assays. The top panels **(A)** show adipogenesis (oil red O staining); the middle panels **(B)** show osteogenesis (calcium deposits); the bottom panels **(C)** show chondrogenesis (toluidine blue staining). All microphotographs were taken on day 21 of culture.

Discussion

This study demonstrated that magnetic enrichment of cells expressing CD271 (LNGFR) could be used for the purification of fresh BM MSCs and for the establishment of highly proliferative and multipotential MSC cultures. The effectiveness of this isolation method could be explained by the high degree of specificity and selectivity of the CD271 (LNGFR) marker for human *in vivo* BM MSCs. Similar to the selection with Anti-Fibroblast MicroBeads, isolation of MSCs by selecting for CD271 (LNGFR) expression did not inhibit MSC growth kinetics. In addition, it proved critical for the morphological identification and purification of culture-initiating cells (fresh MSCs). A similar morphology in freshly purified BM MSCs was previously described after selection with Anti-Fibroblast MicroBeads[3] or cell sorting using a combination of STRO-1/VCAM-1[10]. In terms of cell growth kinetics, the choice of expansion medium was more important than the initial MSC isolation step. Similarly, the extent of tri-lineage differentiation from the same

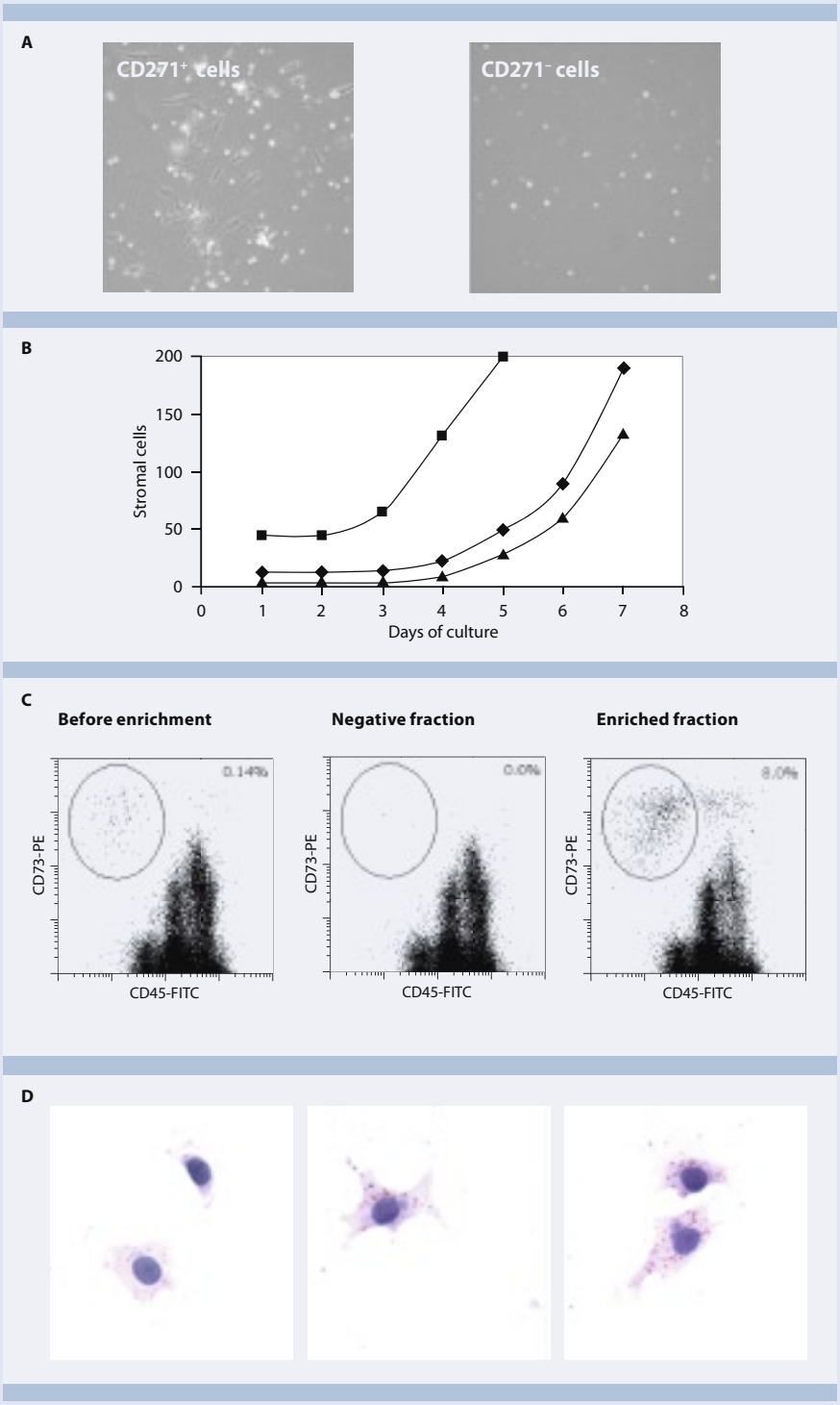

Figure 4 Purification and expansion of marrow stromal cells from the CD271 (LNGFR)–selected cells. **(A)** Microphotographs of adherent cells from the CD271 (LNGFR)–positive cell fraction (left) and the CD271 (LNGFR)–negative cell fractions (right). Small round cells are present in both fractions, whereas dark spindle- and stellate-shaped cells are present exclusively in the positive fraction. **(B)** Proliferation kinetics of CD271 (LNGFR)–isolated stromal cells grown in DMEM/10% FCS showing their exponential growth as early as day three of culture (n=3 donors). **(C)** Enrichment of $CD45^{low}CD73^{+}$ MSCs (circled) in the CD271 (LNGFR)–positive fraction. **(D)** Stromal morphology of sorted $CD45^{low}CD73^{+}$ cells following overnight adherence to plastic (oil red O staining). Tiny droplets of fat in the cytoplasm of many cells could indicate their pre-adipocyte nature.

MSC culture clearly depended on the potency of the differentiation medium. In the area of MSC research, the standardization of isolation, expansion, and differentiation protocols are needed. This study shows that with respect to BM MSC isolation from humans, CD271 (LNGFR)–based methods hold great promise. In addition, these methods can be used for the isolation of stromal reticular cells in BM diseases (such as aplastic anemia and myeloma), in which stromal cell function is compromised.[11,12] Further work is needed to test the utility of CD271 (LNGFR) as a marker of MSCs in other tissues in both humans and in other species, but our data clearly display the potential of CD271 (LNGFR) as a selection marker of proliferative and multipotential MSCs from BM.

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MSC isolation and analysis

CD146—a marker for the isolation of MSCs from the perivascular niche

The human CD146 antigen is also known as MUC18, MCAM, Mel-CAM, and S-Endo-1. CD146 is a transmembrane glycoprotein belonging to the immunoglobulin (Ig) superfamily and contains five extracellular Ig-like domains.[1,2]
The CD146 MicroBead Kit was developed for the isolation of CD146$^+$ cells, such as human endothelial cells, umbilical vein endothelial cells (HUVECs), and pericytes from human tissue. CD146 is expressed in endothelial cells, smooth muscle cells, follicular dendritic cells, melanoma cells, and a subpopulation of activated T lymphocytes.[1,3] The CD146$^+$ cell population isolated from umbilical cord[4], lipoaspirate[5], dental pulp[6], or endometrial tissue[7] contains cells with clonogenic fibroblastic colony (CFU-F) activity and multilineage differentiation potential.

References

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Product	Order no.
CD146 MicroBead Kit, human	130-093-596
CD146-FITC, -PE, -APC, -Biotin, pure	130-092-851, 130-092-853, 130-092-849, 130-092-852, 130-092-850

CD105—a marker for the isolation of human MSCs from bone marrow

The CD105 antigen is a receptor for the growth and differentiation factors TGF-β1 and TGF-β3. Isolated CD105$^+$ bone marrow cells show multipotent differentiation *in vitro*[1] and the capacity to form bone *in vivo* without prior cultivation or differentiation[2].
CD105 MicroBeads are suitable for the isolation of MSCs from bone marrow aspirate. CD105 MicroBeads recognize the same antigen as anti-SH2 antibodies[3], the most commonly employed antibodies for MSC definition after culture.

References

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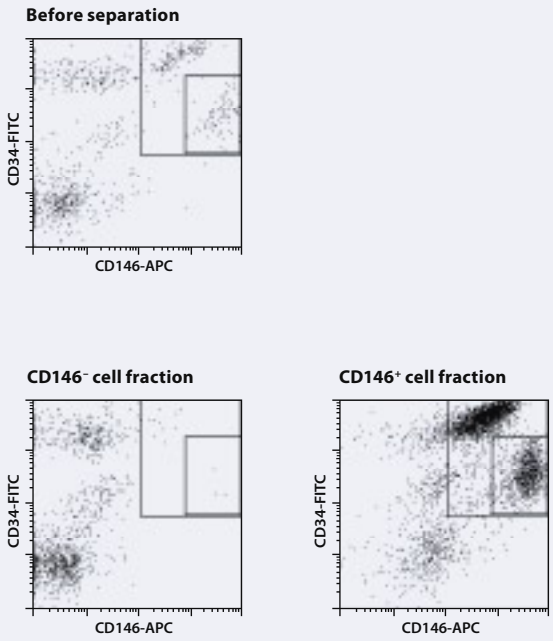
Product	Order no.
CD105 MicroBeads, human	130-051-201



CD146$^+$ cells were separated from lipoaspirate using the CD146 MicroBead Kit, human. Cells were stained with CD146-APC and CD34-FITC and analyzed by flow cytometry.

Enrichment of multipotent adult progenitor cells (MAPCs)

MAPCs are a rare cell population in human and rodent bone marrow. MAPCs copurify with the MSC fraction of bone marrow mononuclear cells. They possess a high expansion capacity and broad differentiation potential.
Human MAPCs were enriched by depleting the mononuclear fraction of CD45$^+$GlyA$^+$ cells from bone marrow and are CD133-positive.[1] Enrichment of mouse MAPCs can be achieved by depletion of CD45$^+$Ter-119$^+$ cells from bone marrow cultures.[2,3]

References

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2. Jiang, Y. *et al.* (2002) Nature 418: 41–49.
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Product	Order no.
CD45 MicroBeads, human	130-045-801
CD235a (Glycophorin A) MicroBeads, human	130-050-501
Anti-Ter-119 MicroBeads, mouse	130-049-901
CD45 MicroBeads, mouse	130-052-301

MSC isolation and analysis

Isolation and analysis of mouse MSCs

Mouse MSC cultures are often heterogeneous populations that are contaminated by lymphohematopoietic $CD34^+CD45^+$ cells[1], hematopoietic stem cells, and macrophages[2]. Therefore, the isolation of MSCs can be a difficult task. Various approaches for the separation of MSCs from other cells have been pursued: contaminating cells have been depleted from MSC cultures according to their expression of CD11b by using CD11b MicroBeads[2], or according to their expression of CD34 and CD45[1], or by using a combination of Anti-Ter-119 and CD45 MicroBeads[3].
Multipotent plastic-adherent fetal stem cells have been positively selected from amniocentesis cultures according to their expression of CD117[4]. These cells showed a pluripotent-like differentiation potential. Mouse MSCs expanded from bone marrow cultures express Sca-1, CD117 (c-kit), and CD105, among other markers.[5]

References

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5. Sun, S. *et al.* (2003) Stem Cells 21: 527–535.

MACS® Products for isolation of mouse MSCs	Strategy for cell isolation	Order no.
Anti-Sca-1 MicroBead Kit (FITC)	Positive selection of $Sca\text{-}1^+$ cells	130-092-529
CD105 MultiSort Kit (PE)	Positive selection of $CD105^+$ cells	130-092-924
CD117 MicroBeads	Positive selection of CD117 $(c\text{-}kit)^+$ cells	130-091-224
Lineage Cell Depletion Kit	Untouched isolation of lin^- cells	130-090-858
CD11b MicroBeads	Depletion of $CD11b^+$ cells	130-049-601
CD45 MicroBeads	Depletion of $CD45^+$ cells	130-052-301
CD90.2 (Thy-1.2) MicroBeads	Positive selection of CD90 $(Thy1.2)^+$ cells	130-049-101
Anti-Ter-119 MicroBeads	Positive selection of $Ter119^+$ cells	130-049-901

MACS Products for analysis of mouse MSCs	Order no.
Anti-Sca-1-FITC, -PE, -APC, -Biotin	130-093-222, 130-093-224, 130-093-223, 130-093-421
CD117-PE, -APC	130-091-730, 130-091-729
CD105-PE, -APC, -Biotin, pure	130-092-929, 130-092-930, 130-092-927, 130-092-926
Anti-Ter-119-FITC, -PE, -APC	130-091-786, 130-091-783, 130-091-788
Lineage Cell Detection Cocktail-Biotin	130-092-613

MSC isolation from virtually any species using MACS® Products for indirect magnetic labeling

MACS® Products for indirect magnetic labeling allow maximum flexibility with regard to the choice of cell surface markers as well as strategies for cell separation. MACS MicroBeads for indirect magnetic labeling recognize either fluorochromes, biotin, or certain immunoglobulin isotypes from mouse, rat, or rabbit. Therefore, the use of virtually any primary antibody for cell sorting is possible. MACS MultiSort Kits permit the positive selection of cells according to two different cell surface markers. MultiSort Kits are available for magnetic labeling of fluorochrome- or biotin-labeled primary antibodies. The table below summarizes a variety of MACS Cell Separations according to different stem cell markers and isolation strategies.

Anti-Fluorochrome MicroBeads

- for magnetic separation of cells stained with fluorochrome-conjugated primary antibodies
- separated cells can be directly used for flow cytometric analysis

Anti-Fluorochrome MicroBeads

Anti-Biotin MicroBeads, Streptavidin MicroBeads

- for magnetic cell sorting with biotinylated primary antibodies
- Anti-Biotin MicroBeads for most efficient magnetic labeling, even of cells with weakly expressed antigens
- Anti-Biotin MicroBeads do not bind to free biotin

Anti-Biotin MicroBeads

Anti-Immunoglobulin MicroBeads

- for magnetic sorting of cells labeled with virtually any antibody from mouse, rat, or rabbit

Anti-Immunoglobulin MicroBeads

MSC isolation and analysis

MACS MicroBeads for indirect magnetic labeling	Order no.
Anti-FITC MicroBeads	130-048-701
Anti-PE MicroBeads	130-048-801
Anti-APC MicroBeads	130-090-855
Anti-Cy5/Anti-Alexa Fluor 647 MicroBeads	130-091-395
Anti-Cy7 MicroBeads	130-091-652
Anti-Biotin MicroBeads	130-090-485
Streptavidin MicroBeads	130-048-101
Rat Anti-Mouse IgG1 MicroBeads	130-047-101
Rat Anti-Mouse IgG2a+b MicroBeads	130-047-201
Rat Anti-Mouse IgM MicroBeads	130-047-301
Goat Anti-Mouse IgG MicroBeads	130-048-401
Mouse Anti-Rat Kappa MicroBeads	130-047-401
Goat Anti-Rat IgG MicroBeads	130-048-501
Goat Anti-Rabbit IgG MicroBeads	130-048-602

MACS MultiSort Kits	Order no.
Anti-FITC MultiSort Kit	130-058-701
Anti-PE MultiSort Kit	130-090-757
Anti-APC MultiSort Kit	130-091-255
Anti-Biotin MultiSort Kit	130-091-256

MACS ® Products for isolation of human MSCs	Order no.
CD117 MicroBead Kit	130-091-332
CD133 MicroBead Kit	130-050-801
CD31 MicroBead Kit	130-091-935
CD34 MicroBead Kit	130-046-702/-703
CD34 MultiSort Kit	130-056-701
CD45 MicroBeads	130-045-801
CD235a (Glycophorin A) MicroBeads	130-050-501
Lineage Cell Depletion Kit	130-092-211

Strategy for the isolation of MSCs	Cell source	Reference
Primary human MSCs		
Positive selection of $CD117^+$ cells	Bone marrow	Huss and Moosmann (2002) Br. J. Haematol. 118: 305–312.
	Amniocentesis cultures	De Coppi *et al.* (2007) Nat. Biotechnol. 25: 100–106.
Positive selection of $CD133^+$ cells	Peripheral blood, bone marrow, cord blood	Tondreau, T. *et al.* (2005) Stem Cells 23: 1105–1112.
	mobilized peripheral blood	Kuçi, S. *et al.* (2008) Cell Prolif. 41, 12–27.
Depletion of $CD45^+CD31^+$ cells	Lipoaspirate/stromal vascular cells	Boquest, A. C. *et al.* (2005) Mol. Biol. Cell 16, 1131–1141. Noer, A. (2006) Mol. Biol. Cell 17: 3543–3556.
Positive selection of $CD34^+$ cells	Lipoaspirate/stromal vascular fraction (SVF)	Astori, G. *et al.* (2007) J. Transl. Med. 5: 55.
Isolation of $CD34^+CD31^-$ cells	Lipoaspirate/stromal vascular fraction (SVF)	Miranville A, *et al.* (2004) Circulation 110: 349–55.
Positive selection of Stro-1^+ cells	Bone marrow	Simmons P. J. and Torok-Storb, B. (1991) Blood 78: 55–62. Gronthos, S. *et al.* (1994) Blood 84: 4164–4173. Gronthos, S. and Simmons P. J. (1995) Blood 85: 929–940. Stewart, K. *et al.* (2003) Cell Tissue Res. 313: 281–290.
	Bone marrow, fetal liver, fetal brain	Airey, J. *et al.* (2004) Circulation 109: 1401–1407.
Positive selection of Stro-1^+CD106 (VCAM)$^+$ cells	Bone marrow	Gronthos, S. *et al.* (2003) J. Cell Sci. 118: 1827–1835.
Positive selection of CD63 (HOP-26)$^+$ cells	Bone marrow	Stewart, K. *et al.* (2003) Cell Tissue Res. 313: 281–290. Zannettino, A. *et al.* (2003) J. Cell. Biochem. 89: 56–66.
Positive selection of $CD49a^+$ cells	Bone marrow	Stewart, K. *et al.* (2003) Cell Tissue Res. 313: 281–290.
Positive selection of CD166 (SB-10)$^+$ cells	Bone marrow	Stewart, K. *et al.* (2003) Cell Tissue Res. 313: 281–290.
Positive selection of GD2 (neural ganglioside)$^+$ cells	Bone marrow	Martinez, C. *et al.* (2007) Blood 109: 4245–4248.
Depletion of $GlyA^+CD45^+$ cells	Bone marrow	Niemeyer, P. *et al.* (2006) Cytotherapy 8: 354–366.
	Maternal blood	O'Donoghue, K. *et al.* (2003) Mol. Hum. Reprod. 9: 497–502.
Primary mouse cells		
Lineage depletion	Bone marrow	Wong, S. H. *et al.* (2007) Stem Cells 25: 1364–1374. Wu, Y. *et al.* (2007) Stem Cells 25: 2648–2659.
Cultured mouse cells		
Positive selection of CD271 (LNGFR/p75NTR)$^+$ cells	Adipose tissue	Yamamoto, N. *et al.* (2007) J. Dermatol. Sci. 48: 43–52.
Depletion of $CD45^+CD34^+$ cells	Bone marrow	Kinnaird, T. *et al.* (2004) Circ. Res. 94: 678–685.

Additional strategies for the isolation of human and mouse MSCs

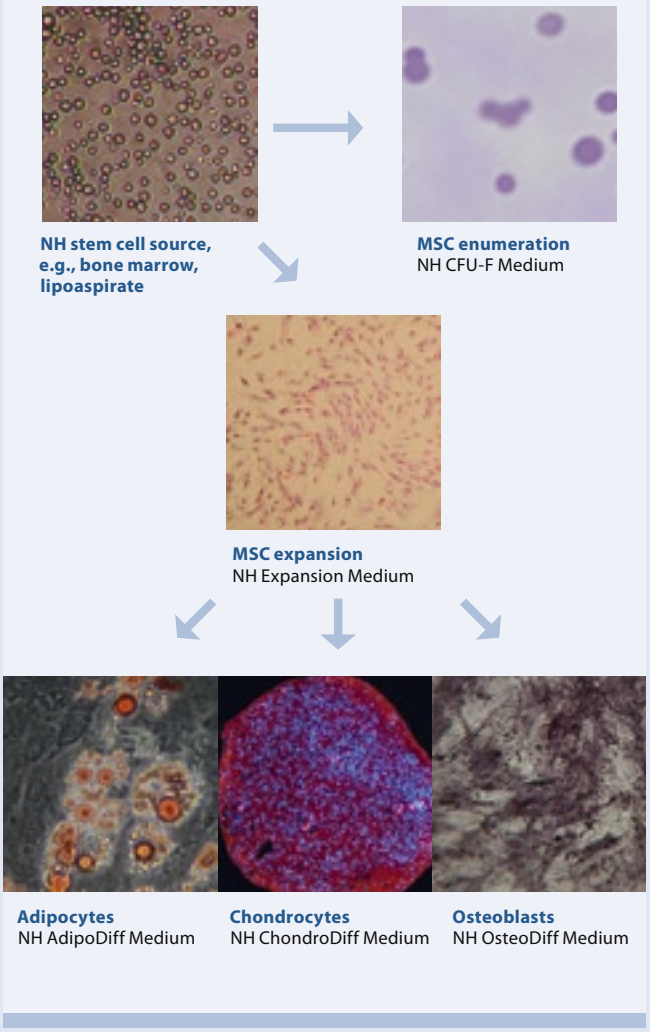
MACS® Cell Culture Products

NH media for the enumeration, expansion, and differentiation of MSCs

MSCs are of great importance to researchers working towards the development of novel tissue regeneration therapies. However, in order to properly evaluate the potential of MSCs completely, it is crucial to establish standardized and reproducible procedures for stem cell isolation and cultivation. MACS® NH Stem Cell Media are optimized for the most convenient enumeration and expansion of nonhematopoietic (NH) stem cells from a variety of sources, including bone marrow aspirate, lipoaspirate, or potentially any tissue or organ within the human body. Miltenyi Biotec also offers media to evaluate the differentiation potential of NH stem cells during *in vitro* cultivation.
Media are available for the reliable and reproducible differentiation of NH stem cells to adipocyte, chondrocyte, or osteoblast lineages. These media can also be used to define the full differentation capacity of an NH stem cell population: MSCs must be able to form all three NH cell lineages.

MACS® NH Media		Order no.
NH CFU-F Medium	24×5 mL	130-091-676
NH Expansion Medium	500 mL	130-091-680
NH AdipoDiff Medium	100 mL	130-091-677
NH ChondroDiff Medium	100 mL	130-091-679
NH OsteoDiff Medium	100 mL	130-091-678

MACS® NH Media enable the differentiation of marrow stromal cells (MSCs) into functional nonhematopoietic (NH) cell types.



CytoMix – MSC, human

The CytoMix – MSC, human (130-093-552) is a composition of cytokines for the most efficient and reproducible expansion of human MSCs. In combination with the NH Expansion Medium, CytoMix – MSC optimally supports the proliferation of human MSCs, especially after separation, e.g., according to CD271 or MSCA-1 (W8B2) expression using MACS® Technology.

Basic media and cytokines for stem cell culture

Miltenyi Biotec offers cell culture media for a broad spectrum of applications. In addition, high-quality recombinant proteins and growth factors are available, that are well-suited for various applications such as cell culture, differentiation studies, and biological assays. Selected products are available in a premium-grade format with excellent purity and high, well-defined activity as well as in research-grade quality.

MACS Basic Culture Media		Order no.
DMEM	500 mL	130-091-437
DMEM with stable glutamine	500 mL	130-091-438
RPMI 1640	500 mL	130-091-440
RPMI 1640 with stable glutamine	500 mL	130-091-439

MACS® Cytokines and Growth Factors
Human: BDNF; BMP-2; EGF; EG-VEGF; FGF-2; FGF-4; Flt3-Ligand; G-CSF; GM-CSF; HGF; IL-3; IL-6; IL-11; PDGF-AA; PDGF-AB; PDGF-BB; SCF; SDF-1α; TGF-α; TGF-β1; TGF-β3; TNF-α; VEGF (121 aa); VEGF (165 aa)
Mouse: EGF; Flt3-Ligand, G-CSF; GM-CSF; IL-3 (135 aa); IL-6; SCF; TNF-α; VEGF (164 aa); VEGF (165 aa);
Rat: VEGF-C; VEGF-C (C152S)

For a complete list of available cytokines, please visit:
http://www.miltenyibiotec.com/cytokines

Molecular biology products and services

Stem cell differentiation tracking by gene expression profiling

MACSmolecular provides a highly innovative range of products and services with a strong focus on gene expression profiling. Particularly when isolating stem cells, sensitive downstream analyses are required.

One-step mRNA isolation and in-column cDNA synthesis

Premium mRNA is isolated within 15 minutes directly from cells or tissues. The µMACS™ One-step cDNA Kit combines efficient magnetic isolation of mRNA with revolutionary in-column cDNA synthesis. Purified cDNA can be generated from just a few to as many as 10^7 cells.

PIQOR™ Stem Cell Microarray

The PIQOR™ Stem Cell Microarray comprises 942 relevant marker genes for human stem cells and their differentiation. It is available as a convenient microarray kit* or within the scope of the microarray service**. Gene expression experiments allow for the quality control of different stem cell types, comparison between different stages of differentiation, as well as the optimization of differentiation protocols.

SuperAmp™ Service ***

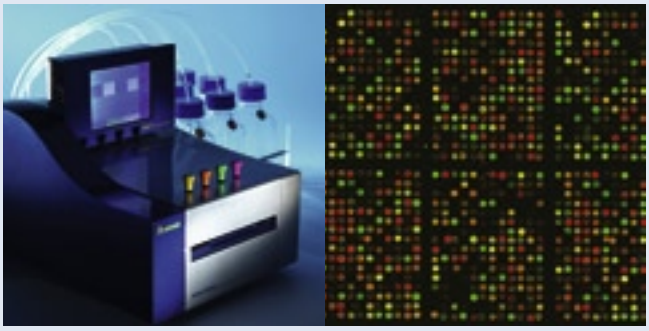
When the number of stem cells for analysis is low, Miltenyi Biotec offers the ideal solution for gene expression profiling needs. The SuperAmp™ Service (available as an extension of the PIQOR™ Microarray Service) can reliably amplify mRNA million-fold from as little as one cell. The service is ideal for stem cells sorted with MACS® Technology, flow cytometry, or even from laser capture microdissected tissue.

miRXplore™ Kits and Services

Explore microRNA expression in human and mouse stem cells with the new miRXplore™ Microarray Kits and Services. Designed in collaboration with experts at the Rockefeller University[1], the microarray covers more than 2700 human, mouse, rat, and viral miRNA sequences and possess rigorous internal control system. Sequences differing by just one oligonucleotide can be reproducibly detected and re-ratios calculated with the use of the proprietary miRXplore Universal Reference.

Reference

1. Landgraf, P. *et al.* (2007) Cell 129: 1401–1414.



a-Hyb™ Hybridization Station

mRNA isolation/cDNA synthesis		
µMACS™ mRNA Isolation Kit	Small Scale Large Scale For Total RNA	130-075-201 130-075-101 130-075-102
µMACS™ mRNA Isolation Starting Kit		130-075-202
µMACS™ One-step cDNA Kit		130-091-902
µMACS™ One-step cDNA Starting Kit		130-091-989

PIQOR™ Microarray Kit *	
PIQOR™ Stem Cell Microarray Kit, antisense	
4 Microarrays	130-092-033
8 Microarrays	130-092-034

PIQOR™ Microarray Service **	
Service Stem Cell Microarray Plus Amplification	160-000-765

SuperAmp™ Amplification ***	
SuperAmp™ Service (per sample)	160-000-936

miRXplore™ Microarray Kit *	
4 Microarrays	130-093-254
8 Microarrays	130-093-272

miRXplore™ Microarray Services	
miRXplore™ Microarray Service	160-001-143
miRXplore™ Universal Reference Service	160-001-161
miRXplore™ Additional Total RNA Extraction	160-001-162

* PIQOR™ Microarray Kits are not available in the US and Canada.
** Microarray Service includes all experimental steps from RNA isolation to primary data analysis. Final data are returned including an extensive written report. Further Bioinformatics Services, such as pathway or cluster analysis, are also available.
*** In combination with the Microarray Services only. The SuperAmp Service is not available for microRNA amplification

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