



Miltenyi Biotec

Stem cell competence

ESC and iPS cell research

Cell separation

Cell analysis

Expression profiling

The preferred choice for stem cell separation

Benefits of MACS® Technology at a glance:

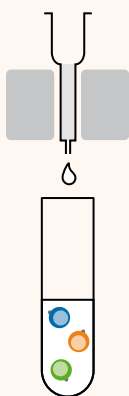
- Fast—cell separation takes less than one hour
- Gentle—separated cells remain viable and functional
- Flexible—both labeled and unlabeled fractions can be obtained with excellent purity and high recovery
- Easy separation of large cell numbers—up to 10⁹ labeled cells per column

MACS Technology



Magnetic labeling

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



Magnetic separation

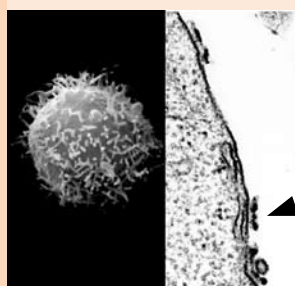
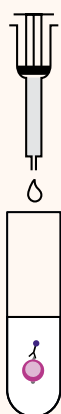
Labeled and unlabeled cells are separated on a MACS Column placed in the magnetic field of a MACS Separator. The flow-through can be collected as the non-magnetic, unlabeled cell fraction.



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.



MACS MicroBeads are nano-sized particles and are barely detectable by scanning electron microscopy. The micrograph shows a lymphocyte isolated by positive selection (left). Transmission electron micrograph of an isolated lymphocyte with MicroBeads (arrow) on the cell surface (right). (Courtesy of Prof. Groscurth, Zürich, CH.)

Pluripotent stem cells

Embryonic stem cells (ESCs) and induced pluripotent stem (iPS) cells have the capacity to self-renew and to differentiate into all cell types of the body in human and mouse. They promise an essentially unlimited supply of specific cell types for basic research, drug testing, and possibly for future transplantation therapies.

To investigate these cells it is crucial to use homogenous cell populations. MACS® Technology enables the isolation of numerous particular cell types and subsets at high purity.

MACS® Technology—the gold standard in cell separation

MACS® Technology is based on MACS MicroBeads, MACS Columns, and MACS Separators—strong permanent magnets. MicroBeads are superparamagnetic particles coupled to specific antibodies.

Target cells can be magnetically isolated by positive selection using specific cell surface antigens or by depletion of unwanted cells in order to obtain untouched cells. Furthermore, these two separation strategies can easily be combined to provide greater flexibility for the sequential sorting of complex subpopulations of cells.

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

- Optimal recovery and high purity with MACS Columns
- Gentle to cells
- Flexible manual cell separation system
- Automated cell separation with autoMACS™ Pro Separator



MidiMACS™ Separator



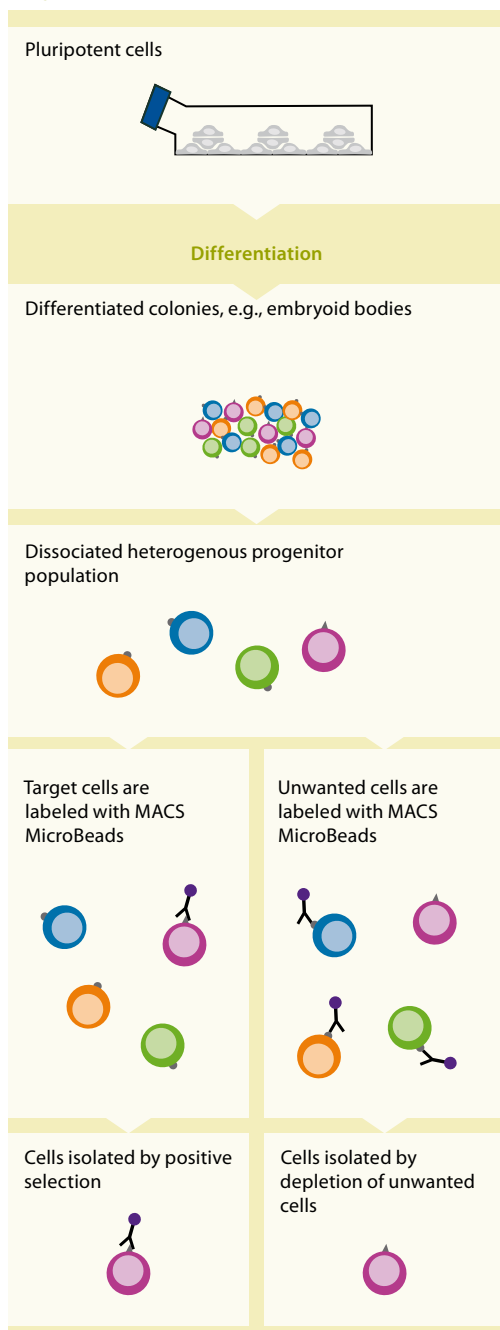
autoMACS™ Pro Separator

MACS® MicroBeads for indirect magnetic labeling

For maximum flexibility, indirect magnetic labeling with MACS® MicroBeads allows the use of any primary antibody. Monoclonal or polyclonal primary antibodies can be either unconjugated, biotinylated, or fluorochrome-conjugated.

Human ESCs and iPS cells

Isolation of ESC- or iPS cell-derived progenitor cells



Differentiated cells of interest can be isolated by positive selection or by depletion of unwanted cells.

References

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- Chen, T. *et al.* (2007) *Stem Cells* 25: 392–401.
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Human ESCs and iPS cells can differentiate *in vitro* into virtually any cell type. MACS® Technology allows the separation of the cells at different time points during differentiation. Target cells can be isolated by positive selection if the cells express a specific surface marker that distinguishes them from other cells. If there is no suitable marker for a particular cell type, depletion of unwanted cells can be a useful alternative for the isolation of the desired cells.

Hematopoietic stem cells (HSCs)

HSCs can be isolated from differentiated ESCs by using, for example, the following cell surface markers:

- CD34¹⁻⁶
- CD34 in combination with CD43⁷
- CD133⁵

The separated HSCs can be further differentiated into:

- CD45⁺ colony-forming hematopoietic progenitor cells (HPCs)^{2,3},
- HPCs that are capable of engrafting primary as well as secondary fetal sheep recipients⁴,
- dendritic cells¹,
- lymphoid (B and natural killer cells) as well as myeloid (macrophages and granulocytes) lineages⁸,
- T lineage⁵,
- erythroid cells, myeloid cells, and megakaryocytes⁶.

For the isolation and analysis of the differentiated immune cells, Miltenyi Biotec offers a wide range of products. For further information, please refer to our website at www.miltenyibiotec.com.

Endothelial progenitor cells (EPCs)

EPCs can be isolated from ESCs by using, for example, the following cell surface markers:

- CD34^{2,3,10}
- CD31

The separated EPCs can be further differentiated into:

- smooth muscle cells and endothelial cells¹⁰,
- a population of endothelial cells that are positive for CD105 (endoglin), von Willebrand factor, CD31, VE-cadherin, CD309 (VEGFR-2/KDR), Tie-2, EphB4, and ephrinB2^{2,3},
- blood vessels *in vivo*².

Early neural cell populations

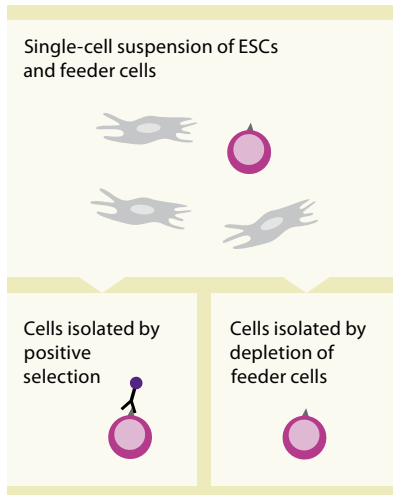
These cells have been enriched with FORSE-1 antibodies and MicroBeads for indirect magnetic labeling⁹.

Miltenyi Biotec also offers numerous products for the enrichment of various neural cell types that can be derived from ESCs, such as:

- CD133⁺ neural stem cells,
- PSA-NCAM⁺ neuronal-restricted precursors,
- A2B5⁺ glial-restricted precursors,
- CD11b⁺ microglial cells,
- CD271 (LNGFR, p75 NTR)⁺ Schwann cells and motor neurons.

Human ESCs and iPS cells

Isolation of ESCs from feeder cell cultures



ESCs can be separated from feeder cells by positive selection of ESCs or by depletion of the feeder cells.

Removal of feeder cells

ESCs and iPS cells are often cultured in the presence of supporting feeder cells, e.g., fibroblasts. These feeder cells can interfere with downstream applications, such as the analysis of transcription, translation, karyotype, and epigenetic status. MACS® Technology enables the separation of ESCs or iPS cells from feeder cells.

Positive selection of human ESCs

There are several options for the separation of ESCs from feeder cells using a positive selection strategy:

- Separation from human multipotent mesenchymal stromal cells (MSCs) according to the expression of SSEA-4 was performed by using MicroBeads for indirect magnetic labeling.¹
- Separation from mouse or human fibroblasts may be achieved by using CD326 (EpCAM) MicroBeads, human.
- Separation from mouse or human fibroblasts according to the expression of SSEA-3 may be achieved by using MicroBeads for indirect magnetic labeling.

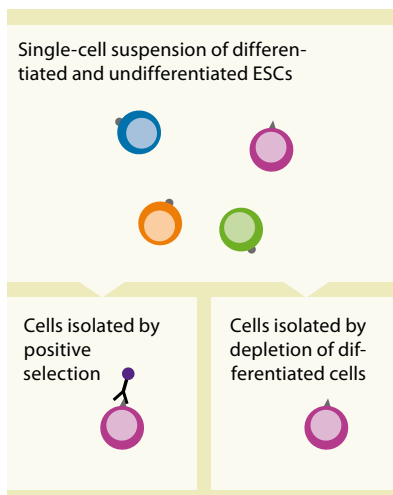
Depletion of feeder cells

- Human fibroblasts can be removed from ESCs and iPS cells by using Anti-Fibroblast MicroBeads, human.
- Any other type of feeder cells can be removed by depletion, if the feeder cells express a surface marker that is not expressed by the ESCs or iPS cells.

Reference

1. Cheng, L. *et al.* (2003) *Stem Cells* 21: 131–142.

Isolation of undifferentiated ESCs



Undifferentiated ESCs can be separated from differentiated cells by positive selection of ESCs or by depletion of the differentiated cells.

Enrichment of undifferentiated, pluripotent ESCs

ESCs tend to differentiate in culture. However, especially for differentiation experiments and for microRNA or gene expression studies it is crucial to obtain homogenous pluripotent cultures. MACS Technology is a straightforward tool for the enrichment of pluripotent stem cells, either by depletion of differentiated cells or by positive selection according to the expression of pluripotency-associated markers, such as SSEA-3, SSEA-4, TRA-1-60, or TRA-1-81.

Positive selection

Positive selection according to the expression of SSEA-4 using MicroBeads for indirect magnetic labeling is a proven strategy for the isolation of pluripotent cells:

- SSEA-4⁺ cells were enriched to 94% from a mixed culture containing 8.6% SSEA-4⁺ cells.¹
- A culture containing 85% SSEA-4⁺ ESCs was further enriched to obtain 99% SSEA-4⁺ cells.²

Depletion

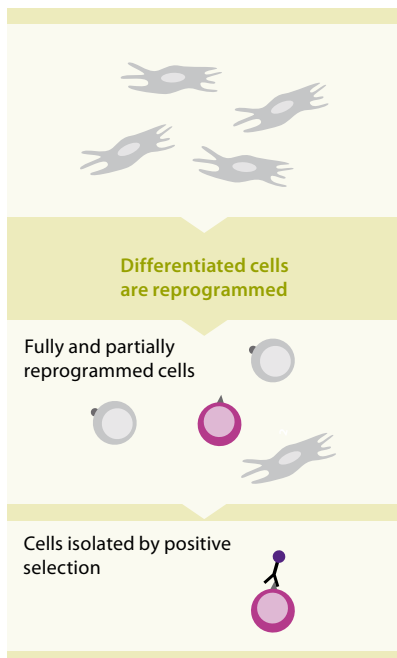
Alternatively, the depletion of differentiated cells is recommended, for example, via CD15 (SSEA-1).

References

1. Cheng, L. *et al.* (2003) *Stem Cells* 21: 131–42.

2. Schulz, T. C. *et al.* (2004) *Stem Cells* 22: 1218–1238.

Isolation of iPS cells



Successfully reprogrammed, pluripotent stem cells can be isolated by positive selection.

Efficient generation of iPS cells

During the generation of iPS cells the up-regulation of pluripotency markers, such as SSEA-3 and SSEA-4, occurs at different time points and at variable expression intensities. To obtain only fully reprogrammed cells showing high expression of pluripotency markers, positive selection, e.g., via SSEA-3 is useful. The fast magnetic isolation of SSEA-3⁺ cells by MACS[®] Technology may enhance the generation of iPS cells by:

- synchronization of the culture,
- removal of transformed cells,
- exclusion of remaining differentiated cells.

Mouse ESCs and iPS cells

Pluripotent ESCs and iPS cells

These cell types express the surface marker SSEA-1. SSEA-1⁺ cells can be isolated by using MicroBeads for indirect magnetic labeling¹⁻³, e.g., to eliminate feeder cells. Anti-SSEA-1 (CD15) MicroBeads for direct magnetic labeling will soon be available. These MicroBeads can also be used to remove unwanted remaining pluripotent cells in ESC-derived differentiation cultures.

Prominin-1 expression is restricted to peripheral regions of ESC colonies and seems to be expressed by ESCs that just started to differentiate.⁴ Therefore, the Anti-Prominin-1 MicroBeads can be used to obtain pluripotent cells by depleting the early differentiated cells. The Annexin V MicroBead Kit can be used to deplete apoptotic cells from ESC cultures.⁵

The generation of iPS cells can be enhanced by enrichment of SSEA-1⁺ cells.⁶ During the reprogramming of mouse fibroblasts to iPS cells, SSEA-1 is one of the first pluripotency markers that is expressed.⁷

ESC- or iPS cell-derived progenitors

Differentiation of ESCs towards defined cell populations, e.g., for *in vivo* tissue regeneration experiments, is one of the major goals in mouse stem cell research. MACS[®] Technology can be used to deplete unwanted cells during the differentiation process or to enrich the cells of interest via positive selection. CD117 MicroBeads have been used to separate ESC-derived HSCs.⁸ Large numbers of endothelial cells with high purity were obtained using Sca-1⁺ cells, immunomagnetically isolated from pre-differentiated ESCs.⁹ Additionally smooth muscle cells can be differentiated from ESCs via the isolation of Sca-1⁺ cells.¹⁰

References

1. Durcova, G. *et al.* (1998) *J. Reprod. Dev.* 44:85–89.
2. Cui, L. *et al.* (2004) *J. Histochem. Cytochem.* 52: 1447–1457.
3. Shin, S. *et al.* (2007) *Stem Cells Dev.* 16:131–141.
4. Kania, G. *et al.* (2005) *Stem Cells* 23: 791–804.
5. Bashamboo, A. *et al.* (2006) *J. Cell Sci.* 119: 3039–3046.
6. Stadtfeld, M. *et al.* (2008) *Cell Stem Cell.* 6: 230–240.
7. Brambrink, T. *et al.* (2008) *Cell Stem Cell.* 2:151–159.
8. Verda, L. *et al.* (2008) *Stem Cells* 26 :381–386.
9. Xiao, Q. *et al.* (2006) *Arterioscler. Thromb. Vasc. Biol.* 26: 2244–2251.
10. Zampetaki, A. *et al.* (2007) *Am. J. Physiol. Cell Physiol.* 293: C1226–C1238.

MACS® Products for ESC and iPS cell research—product overview

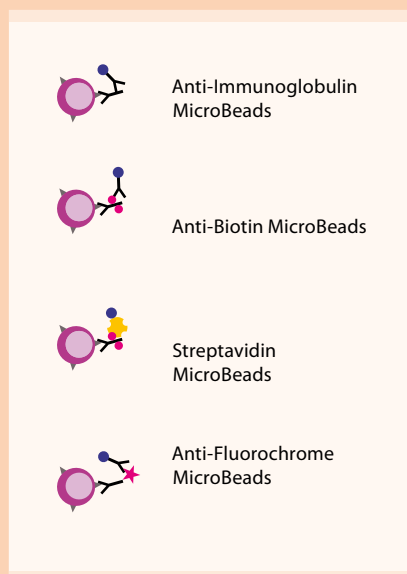
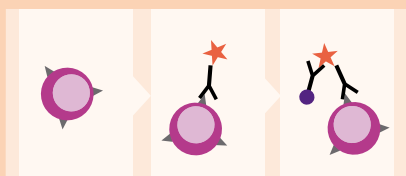
MACS Cell Separation products

Product	Order no.
Annexin V MicroBead Kit	130-090-201
Anti-A2B5 MicroBeads, human, mouse, rat	130-093-388
Anti-Fibroblast MicroBeads, human	130-050-601
Anti-Prominin-1 MicroBeads, mouse	130-092-333
Anti-PSA-NCAM MicroBeads, human, mouse, rat	130-092-966
Anti-Sca-1 MicroBead Kit (FITC), mouse	130-092-529
Anti-SSEA-1(CD15) MicroBeads, mouse	Coming soon 130-094-530
CD31 MicroBead Kit, human	130-091-935

CD34 MicroBead Kit, human	130-046-702
CD34 MicroBead Kit, human (for 10 ¹⁰ total cells)	130-046-703
CD34 MultiSort Kit, human	130-056-701
CD105 MicroBeads, human	130-051-201
CD105 MultiSort Kit (PE), mouse	130-092-924
CD117 MicroBeads, mouse	130-091-224
CD117 MicroBead Kit, human	130-091-332
CD133 MicroBead Kit, human	130-050-801
CD326 (EpCAM) MicroBeads, human	130-061-101
Indirect CD133 MicroBead Kit, human	130-091-895
Lineage Cell Depletion Kit, human	130-092-211
Lineage Cell Depletion Kit, mouse	130-090-858

MACS® Technology

MACS Products for indirect magnetic labeling



Indirect labeling is a convenient alternative when direct magnetic labeling is not possible or if established primary antibodies shall be used. As a primary antibody, a specific monoclonal or polyclonal antibody, directed against any cell surface marker can be used for this labeling strategy. The primary antibody can either be unconjugated, biotinylated, or fluorochrome-conjugated. The magnetic labeling is then achieved with Anti-Immunoglobulin MicroBeads, Anti-Biotin MicroBeads, or Anti-Fluorochrome MicroBeads. Alternatively, a cocktail of primary antibodies can be used to concurrently isolate or deplete a number of cell types. Indirect labeling is also the method of choice when dimly expressed markers serve as targets, because it amplifies the magnetic labeling.

- Enrichment or depletion of virtually any cell type
- For conjugated or unconjugated primary antibodies
- Compatible with fluorescent staining for flow cytometric or microscopic analysis

Anti-Fluorochrome MicroBeads

- For magnetic separation of cells stained with fluorochrome-conjugated primary antibodies
- Separated cells can directly be analyzed by flow cytometry or microscopy

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-Immunoglobulin MicroBeads

- For magnetic sorting of cells labeled with antibodies from various sources, for example, mouse, rat, or rabbit

MACS® Products for indirect magnetic labeling

Product	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
MultiSort Kits (releasable, for multiple cell sorting)	
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 Cell Culture products

	Product	Order no.
Cytokines*	Human: Activin A, BDNF, BMP-2, BMP-6, BMP-7; EGF, FGF-2 (basic FGF), FGF-4, Flt3-Ligand, G-CSF, GM-CSF, IGF-1, IL-6, M-CSF, HGF, NGF-β, SCF, TGF-β1, TGF-β2, TGF-β3, VEGF	
	Mouse: EGF, G-CSF, GM-CSF, SCF, VEGF	
Media**	HSC-CFU complete with Epo, human – 100 mL or 24x3 mL	130-091-280 130-091-278
	HSC-CFU complete w/o Epo, human – 100 mL or 24x3 mL	130-091-277 130-091-276
	HSC-CFU lite with Epo, human – 100 mL or 24x3 mL	130-091-281 130-091-282
	HSC-CFU basic, human – 80 mL	130-091-275

* Selected cytokines are available in a premium grade format for high, well-defined activity as well as in research grade quality. For a complete list of available cytokines, please visit: www.miltenyibiotec.com/cytokines

** For a complete list of MACS Media, please visit:

www.miltenyibiotec.com/media

MACS Cell Analysis products

Antibody		Order no.
Anti-A2B5, human, mouse, rat	-PE	130-093-581
	-APC	130-093-582
	pure	130-093-394
Anti-PSA-NCAM, human, mouse, rat	-PE	130-093-274
	-APC	130-093-273
Anti-Sca-1, mouse	-FITC	130-093-222
	-PE	130-093-224
	-APC	130-093-223
	-Biotin	130-093-421
CD15, human***	-FITC	130-081-101
	-PE	130-091-375
	-APC	130-091-371
CD31, human	-FITC	130-092-654
	-PE	130-092-653
	-APC	130-092-652
CD34, human	-FITC	130-081-001
	-PE	130-081-002
	-APC	130-090-954
CD105, mouse	-PE	130-092-929
	-APC	130-092-930
	-Biotin	130-092-927
	pure	130-092-926
CD117 (A3C6E2), human	-PE	130-091-734
	-APC	130-091-733
CD117, mouse	-PE	130-091-730
	-APC	130-091-729
CD133/1 (AC133), human	-PE	130-080-801
	-APC	130-090-826
	-Biotin	130-090-664
	pure	130-090-422
CD133/2 (293C3), human	-PE	130-090-853
	-APC	130-090-854
	-Biotin	130-090-852
	pure	130-090-851
CD326 (EpCAM), human	-FITC	130-080-301
	-PE	130-091-253
	-APC	130-091-254
Lineage Cell Detection Cocktail, mouse	-Biotin	130-092-613

*** also known as SSEA-1; cross-reactive with mouse



a-Hyb™ Hybridization Station

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

mRNA isolation/cDNA synthesis

μMACS™ mRNA Isolation Kit – Small Scale # 130-075-201

μMACS™ mRNA Isolation Kit – Large Scale # 130-075-101

μMACS™ One-step cDNA Kit # 130-091-902

PIQOR™ Microarray Service *

Service Stem Cell Microarray Plus Amplification # 160-000-765

SuperAmp™ Amplification **

SuperAmp™ Service (per sample) # 160-000-936

Stem cell differentiation tracking by gene expression profiling

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

microRNA expression profiling—miRXplore™ Kits and Services

Explore microRNA expression in human and mouse stem cells with the miRXplore™ Microarray Kits and Services. Designed in collaboration with experts at the Rockefeller University¹, the microarray covers more than 2000 human, mouse, rat, and viral microRNA 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, a synthetic microRNA pool.

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 10⁷ cells.²

PIQOR™ Stem Cell Microarray Service*

The PIQOR™ Stem Cell Microarray comprises 942 relevant marker genes for human stem cells and their differentiation. 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.

References

1. Landgraf, P. *et al.* (2007) *Cell* 129: 1401–1414.
2. Mack *et al.* (2007) *Cytometry A* 71: 404–409.

* 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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