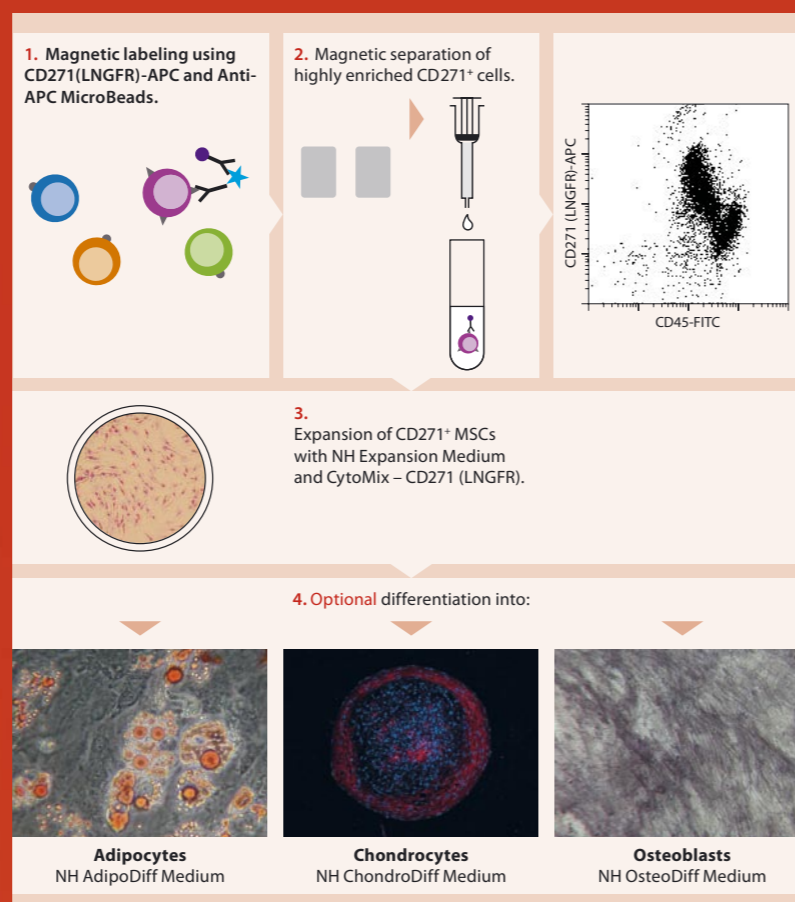


Nonhematopoietic (NH) stem cells from the bone marrow, termed marrow stromal cells (MSCs), show a high capacity to differentiate into nonhematopoietic tissues. Therefore, these adult stem cells may potentially be applied for the future treatment of inherited or degenerative disorders, tissue injuries, or in conjunction with gene therapy. The growing interest in MSCs as potential therapeutic tools necessitates the definition of standardized and reproducible experimental conditions. CD271, also known as low-affinity nerve growth factor receptor (LNGFR), is the most suitable marker for the isolation of MSCs directly from human bone marrow.<sup>1,2</sup> MSCs from other sources have also been shown to be CD271<sup>+</sup>, such as those from the synovial fluid of arthritic joints.<sup>3</sup> Positive selection of CD271 (LNGFR)<sup>+</sup> MSCs resulted in a homogeneous MSC population, whereas no colony forming unit-fibroblast (CFU-F) activity was found in the CD271 (LNGFR)-negative fraction.<sup>4</sup> Furthermore, MSCs isolated by CD271 were shown to have a > 100-fold higher proliferative capacity compared with MSCs isolated by plastic adherence.<sup>4</sup>

CD271<sup>+</sup> separated and expanded MSCs maintain their multilineage differentiation potential<sup>2,4</sup>, displaying the plasticity to differentiate into adipocytes, chondrocytes<sup>5</sup>, osteoblasts<sup>(2,4,5)</sup>, and endothelial cells<sup>6</sup>.

- 1) Jones, E. *et al.* (2004) (Abstract) 4th Annual Conference on mesenchymal and nonhematopoietic stem cells.
- 2) Jones, E. *et al.* (2004) (Abstract) ASH (2337).
- 3) Jones, E. *et al.* (2004) Arthritis & Rheumatism 50: 817–827.
- 4) Quirici, N. *et al.* (2002) Exp. Hematol. 30: 783–791.
- 5) Jones, E. *et al.* (2002) Arthritis & Rheumatism 46: 3349–3360.
- 6) Quirici, N. *et al.* (2004) (Abstract) ASH (2333).



© 2006 Miltenyi Biotec GmbH. Printed in Germany.



# MSC Research Tool Box – CD271 (LNGFR)

For the standardization of human marrow stromal cell (MSC) research.

- Magnetic enrichment using CD271 (LNGFR) – the most suitable marker for isolating a defined MSC population.
- More than 100-fold enrichment of MSCs vs. plastic adherence.
- Optimized MSC cultivation and expansion.
- Enhanced MSC proliferation potential.
- Differentiation potential maintained throughout cultivation.
- Convenient, all-in-one tool box.

## MSC Research Tool Box – CD271 (LNGFR)

130-092-291

Components:	Quantity/Size
Isolation	CD271 (LNGFR)-APC Anti-APC MicroBeads FcR Blocking Reagent
Expansion	NH Expansion Medium CytoMix – CD271 (LNGFR)

## Related products

Order no.

CD271 (LNGFR) MicroBead Kit	130-092-283
NH CFU-F Medium	130-091-676
NH Expansion Medium	130-091-680
NH AdipoDiff Medium	130-091-677
NH ChondroDiff Medium	130-091-679
NH OsteoDiff Medium	130-091-678

For a complete list of cell and molecular analysis products and services, please refer to our website at [www.miltenyibiotec.com](http://www.miltenyibiotec.com).

Unless otherwise specifically indicated, all Miltenyi Biotec products and services are for research use only and not for diagnostic or therapeutic use. MACS is a registered trademark of Miltenyi Biotec GmbH.

## Miltenyi Biotec

**Miltenyi Biotec GmbH**  
Friedrich-Ebert-Straße 68  
51429 Bergisch Gladbach  
Germany  
Phone +49 2204 8306-0  
Fax +49 2204 85197  
macs@miltenyibiotec.de

**Miltenyi Biotec Inc.**  
12740 Earhart Avenue  
Auburn CA 95602, USA  
Phone 800 FOR MACS,  
+1 530 888 8871  
Fax +1 530 888 8925  
macs@miltenyibiotec.com

**Miltenyi Biotec Pty. Ltd.**  
(Australia)  
Phone +61 02 8877 7400  
macs@miltenyibiotec.com.au

**Miltenyi Biotec B. V. (Benelux)**  
macs@miltenyibiotec.nl  
Customer service, Netherlands  
Phone 0800 4020120  
Customer service, Belgium  
Phone 0800 94016

Customer service, Luxembourg  
Phone 800 24971  
**Miltenyi Biotec Shanghai Office**  
Phone +86 21 6235 1005  
macs@miltenyibiotec.com.cn

**Miltenyi Biotec (France)**  
Phone +33 1 56 98 16 16  
macs@miltenyibiotec.fr

**Miltenyi Biotec S.r.l. (Italy)**  
Phone +39 051 646 0411  
macs@miltenyibiotec.it

**Miltenyi Biotec K.K. (Japan)**  
Phone +81 3 56 46 8910  
macs@miltenyibiotec.jp

**Miltenyi Biotec Asia Pacific Pte. Ltd. (Singapore)**  
Phone +65 6238 8183  
macs@miltenyibiotec.com.sg

**Miltenyi Biotec S.L. (Spain)**  
Phone +34 91 512 12 90  
macs@miltenyibiotec.es

**Miltenyi Biotec Ltd. (UK)**  
Phone +44 1483 799 800  
macs@miltenyibiotec.co.uk

[www.miltenyibiotec.com](http://www.miltenyibiotec.com)



130-092-586.2

Miltenyi Biotec



# The expansion and differentiation potential of CD271 (LNGFR)<sup>+</sup> marrow stromal cells (MSCs) vs. MSCs isolated by plastic adherence

K. Godthardt, S. Donath, T. Peters-Regehr, S. Deppe, C. Piechaczek, J. Schmitz Miltenyi Biotec GmbH, Bergisch-Gladbach, Germany

## Introduction

In bone marrow (BM), stem and progenitor cells with haematopoietic differentiation potential exist in close proximity to another heterogeneous group of stem cells with nonhaematopoietic (NH) differentiation potential, termed marrow stromal cells (MSC). A subpopulation of these marrow stromal cells with an unknown phenotype can be isolated by plastic adherence (PA). Many attempts have been made to find the most specific antigen for the isolation of

MSCs and include the use of CD105, CD117, CD271 (LNGFR), Stro-1 and D7-Fib, a fibroblast marker. Isolated CD271<sup>+</sup> bone marrow cells, in comparison to plastic adherent bone marrow cells, have been reported to show a one to three log greater expansion of MSCs in culture as well as a greater capacity to differentiate to adipocytes, chondrocytes and osteoblasts. Therefore, CD271 has become one of the most promising markers for MSC isolation.<sup>1,2</sup>

## Methods

MSC isolation and cultivation were performed using the MSC Research Tool Box – CD271 (LNGFR) (Miltenyi Biotec GmbH), containing CD271 (LNGFR)-APC, Anti-APC MicroBeads for cell separation and NH Expansion Medium supplemented with CytoMix – CD271 (LNGFR) for cultivation of isolated cells.

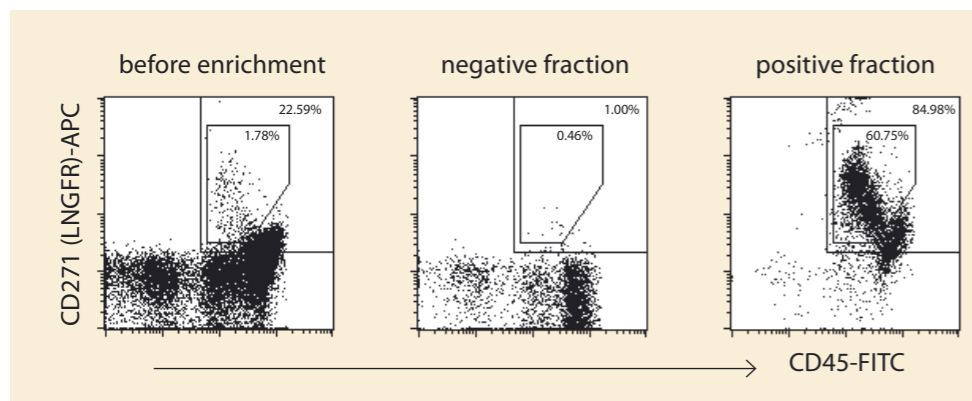
2x10<sup>7</sup> bone marrow mononuclear cells (BM MNC) were separated using CD271 (LNGFR)-APC and Anti-APC MicroBeads. Both the negative and positively-selected fractions were cultivated with NH Expansion Medium, with and without CytoMix – CD271, in order to assess their proliferative capacity. These fractions were then compared to MSCs that had previously been isolated by PA and then expanded using the same cultivation conditions. The phenotype, population doubling (PD\*) and cumulative population doubling (CPD\*\*) levels were determined after

41 days of cultivation using the equation shown below<sup>3</sup>. To assess clonogenic potential, a colony-forming unit fibroblast (CFU-F) assay was performed on 1x10<sup>6</sup> BM MNC, 1x10<sup>6</sup> cells of the negative fraction, and a sample of the positive fraction that had been cultivated in NH Expansion Medium for 14 days. Furthermore, multipotent differentiation potential was investigated by culturing 5x10<sup>4</sup> MSCs in NH AdipoDiff and NH OsteoDiff Media (Miltenyi Biotec GmbH). In order to observe chondrogenic differentiation, a micromass<sup>4</sup> culture with 2x10<sup>5</sup> MSCs in NH ChondroDiff Medium (Miltenyi Biotec GmbH) was also performed. Negative controls were cultured in NH Expansion Medium only.

\* PD for each subcultivation:  $(\log_{10}(NH) - \log_{10}(NI)) / \log_{10}(2)$   
 NI = inoculum number of cells; NH = cell harvest number  
 \*\* CPD for 41 days of culture:  $\sum PD$

## Results

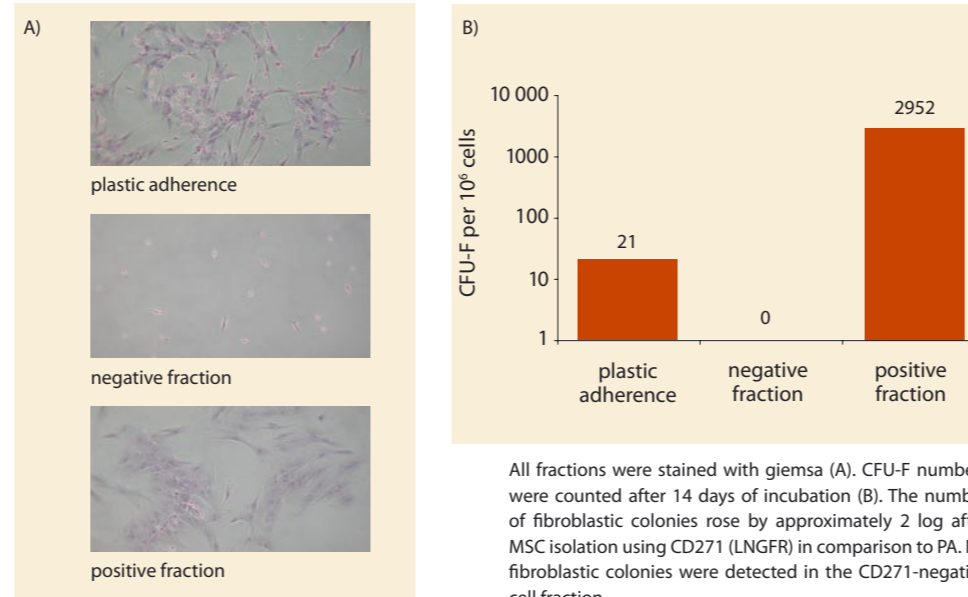
### 1 Positive selection of CD271 (LNGFR)<sup>+</sup> cells from BM MNC



2x10<sup>7</sup> BM MNC were isolated using CD271 (LNGFR)-APC and Anti-APC MicroBeads. Samples of all fractions were labelled with CD45-FITC. PI immunofluorescence and light scatter signals were used for gating viable cells. The positive

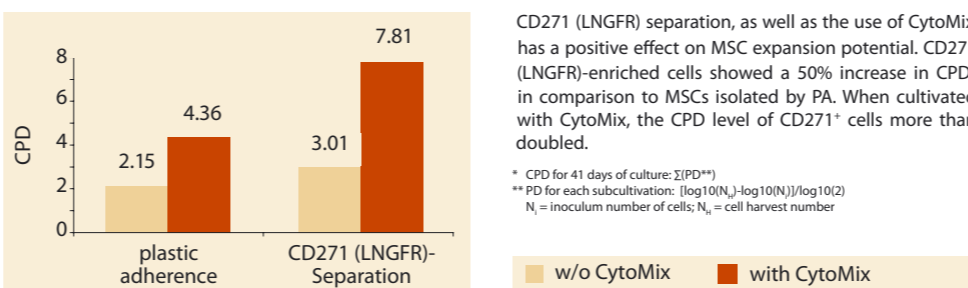
fraction, containing CD271 (LNGFR)<sup>+</sup> cells, showed a purity of 83% ± 4.1% (n=4). This population contained 61% CD271 (LNGFR)<sup>bright</sup>/CD45<sup>dim</sup> cells and 22% CD271 (LNGFR)<sup>dim</sup>/CD45<sup>bright</sup> cells.

### 2 Colony-forming unit fibroblast (CFU-F) assay



All fractions were stained with giemsa (A). CFU-F numbers were counted after 14 days of incubation (B). The number of fibroblastic colonies rose by approximately 2 log after MSC isolation using CD271 (LNGFR) in comparison to PA. No fibroblastic colonies were detected in the CD271-negative cell fraction.

### 3 Expansion of MSCs isolated by CD271 separation vs. plastic adherence and assessment of the CPD

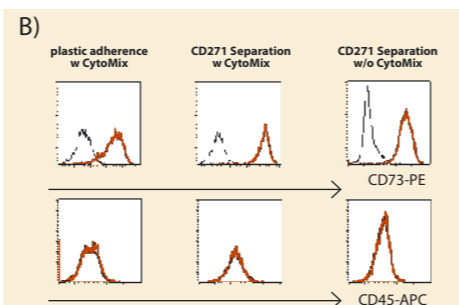


CD271 (LNGFR) separation, as well as the use of CytoMix, has a positive effect on MSC expansion potential. CD271 (LNGFR)-enriched cells showed a 50% increase in CPD\* in comparison to MSCs isolated by PA. When cultivated with CytoMix, the CPD level of CD271<sup>+</sup> cells more than doubled.

\* CPD for 41 days of culture:  $\sum PD$ \*\*  
 \*\* PD for each subcultivation:  $(\log_{10}(N_h) - \log_{10}(N_i)) / \log_{10}(2)$   
 N<sub>i</sub> = inoculum number of cells; N<sub>h</sub> = cell harvest number

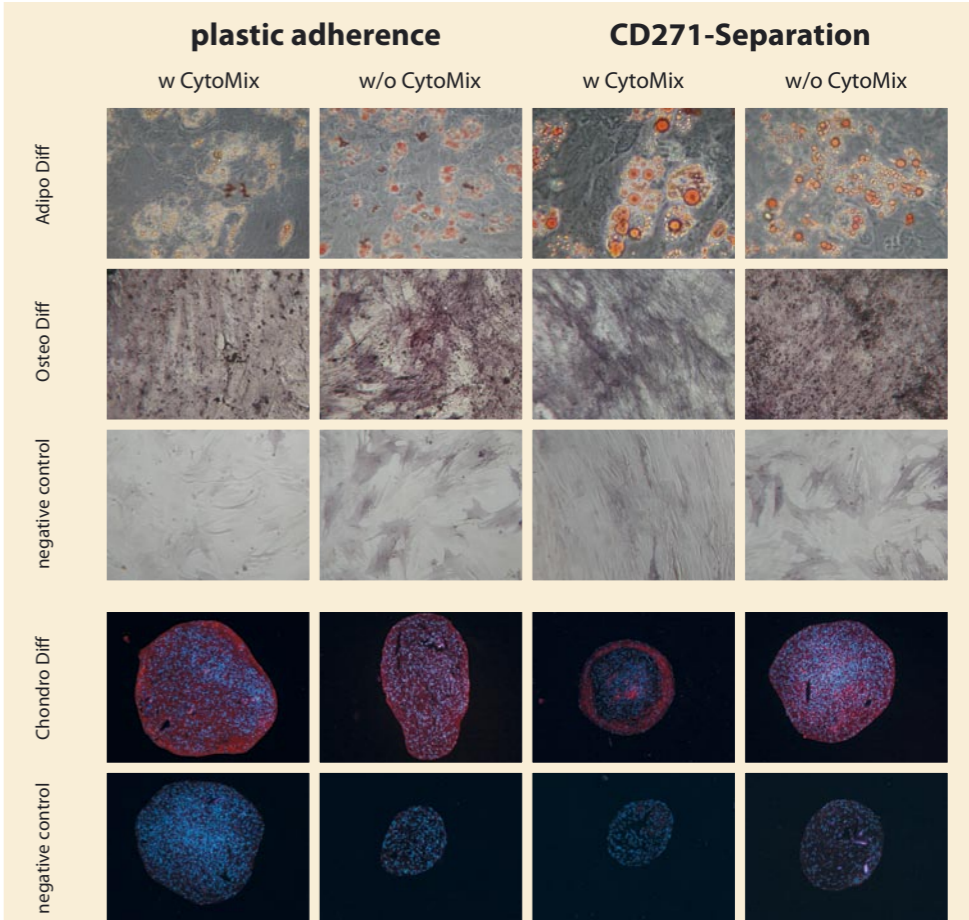
### 4 Phenotyping of *in vitro* expanded MSCs isolated by CD271 separation and plastic adherence

MSC Fraction	CD29	CD44	CD73	CD105	CD166	CD14	CD34	CD45
PA w CytoMix	+	+	+	+	+	-	-	-
CD271 <sup>+</sup> w CytoMix	+	+	+	+	+	-	-	-
CD271 <sup>+</sup> w/o CytoMix	+	+	+	+	+	-	-	-



After 41 days of culture using NH Expansion Medium (with and without CytoMix), MSCs were trypsinized and stained with antibody conjugates for different markers (—) and with the corresponding isotype controls (—). *In vitro* expanded MSCs stain positive for CD29, CD44, CD73, CD105, CD166 and are negative for CD14, CD34, CD45. Independent of whether MSCs were isolated by PA, CD271 separation or cultivated with or without CytoMix, cells matched these criteria after expansion (A). The histograms in B show example stainings of CD73 and CD45 for all separation and cultivation conditions. All cultivated cells are clearly CD73<sup>+</sup> and CD45<sup>-</sup>.

### 5 Differentiation potential of MSCs isolated by CD271 separation vs. plastic adherence



After 18 days of cultivation in NH AdipoDiff Medium, all MSCs showed an increased accumulation of intracellular lipid vacuoles, as revealed by Oil Red-O staining. After 10 days of cultivation in NH OsteoDiff Medium, all MSCs showed a high alkaline phosphatase (AP) activity. Detection of AP activity was performed with Fast BCIP/NBT substrate. After 24 days of cultivation

in NH ChondroDiff Medium, nodules were processed immunohistochemically for the detection of chondrocyte-specific aggrecan. Indirect staining was achieved using an anti-aggrecan antibody and a Rhodamine Red-labeled secondary antibody. Cell nuclei were stained with 4'-6-Diamidino-2-phenylindole (DAPI). No differentiation was seen in the negative controls.

## Conclusion

Highly purified CD271<sup>+</sup> cells show greater expansion potential in the number of fibroblastic colonies formed in comparison to MSCs isolated by plastic adherence. They possess the ability to give rise to adipocytes, chondrocytes and osteoblasts.

Historically, a number of methods that utilize the physical properties of MSCs have been used to isolate these cells from sites at which they reside. However, no physical properties have been uniquely ascribed to MSCs, therefore many different cell types are co-isolated which results in a mixed population of cells. The purification approach embodied in the CD271 (LNGFR)

isolation results in a cell population that contains approximately a 100-fold greater concentration of MSCs compared to conventional methods of isolation. This enables the isolation of a homogeneous and consequently more effective NH stem cell population.

References:  
 1. Jones, E. et al. (2004) (Abstract) ASH (2337).  
 2. Quirici et al. (2002) Experimental Hematology 30: 783–791.  
 3. Christofalo et al. (1998) Proc. Natl. Acad. Sci. USA 95: 10614–10619.  
 4. Johnstone et al. (1998) Exp. Cell Res. 238: 265–272.  
 5. Pittenger et al. (1999) Science 284: 143–146.