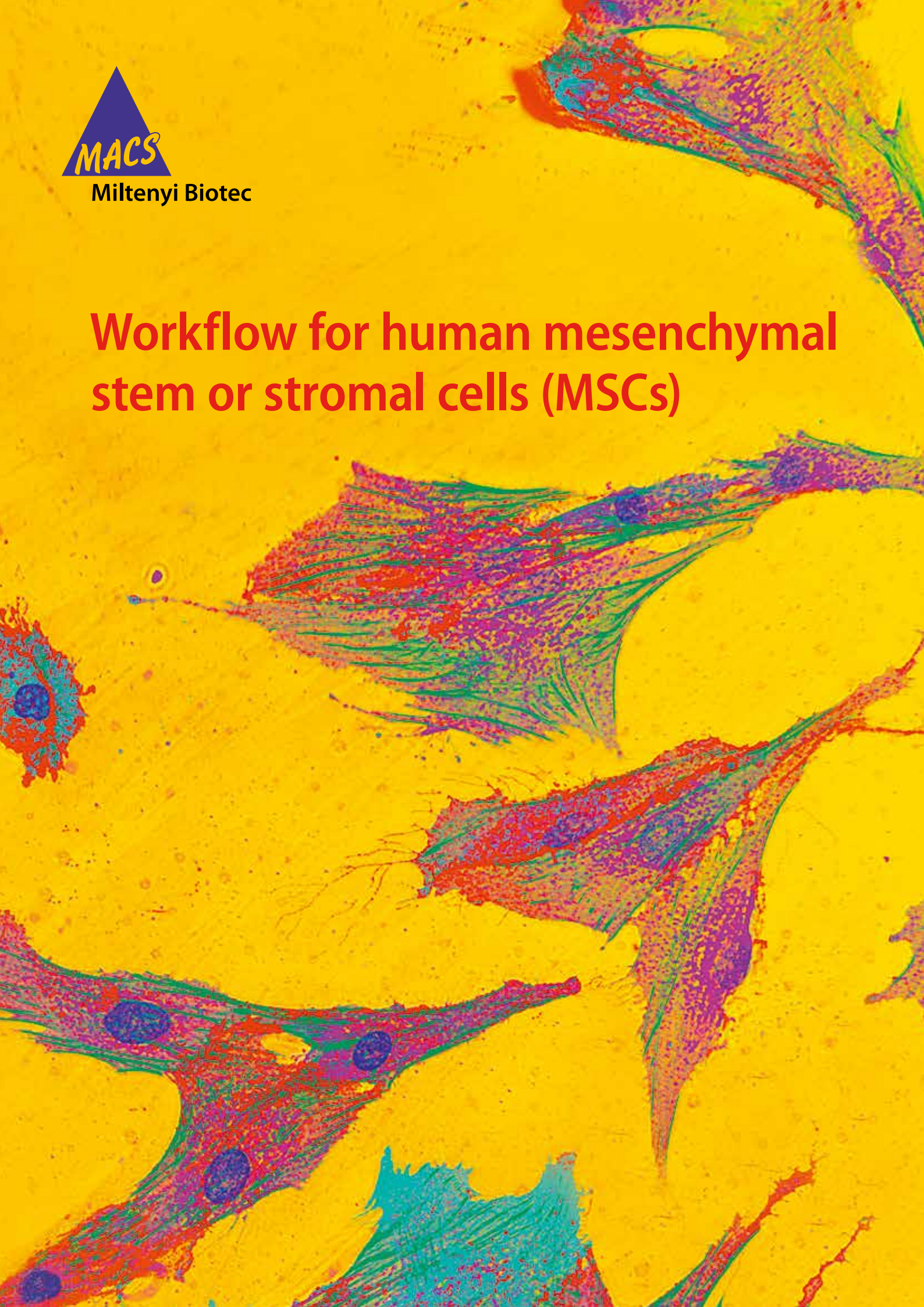
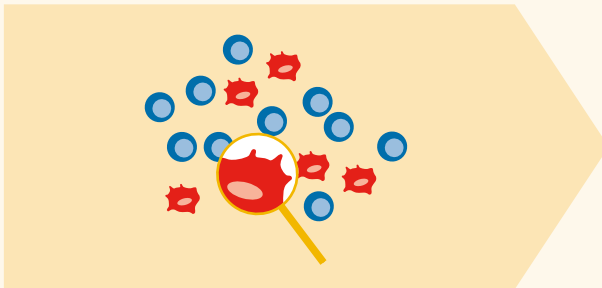


# Workflow for human mesenchymal stem or stromal cells (MSCs)



# Complete workflow for bone marrow-derived mesenchymal stem or stromal cells (MSCs)



## 1. Quality control of bone marrow samples with MSC Enumeration Kit

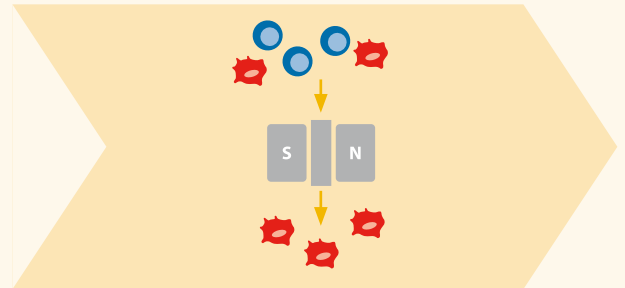
- Standardized identification and quantification of MSCs from fresh bone marrow aspirate (BMA) by flow cytometry
- Only 200 µl bone marrow samples needed per test
- 30 minutes protocol

### Kit includes:

- A cocktail of three fluorochrome-conjugated antibodies including MSC characterization marker CD271 (LNGFR) and exclusion markers CD45 and CD235a
- Additional MSC marker MSCA-1 (W8B2), for the identification of MSCs with high proliferative potential
- A cocktail of four fluorochrome-conjugated antibodies for isotype control
- Cell viability marker 7-AAD
- Red Blood Cell Lysis Solution to remove erythrocytes
- FcR blocking reagent to prevent unspecific binding

Automated and accurate cell count can be performed on MACSQuant® Flow Cytometers.

Products	Order no.
MSC Enumeration Kit, human	130-106-646



## 2. MSC enrichment with magnetic cell separation

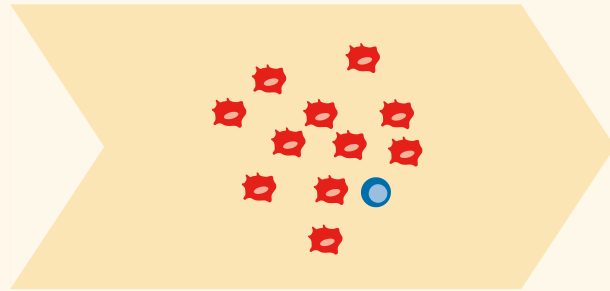
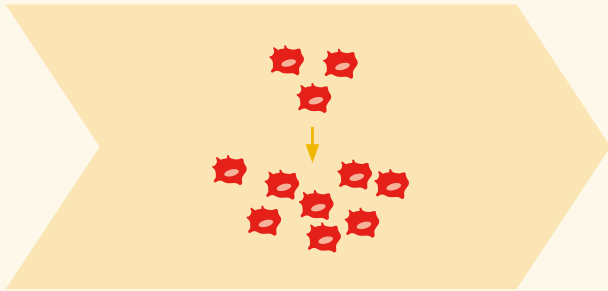
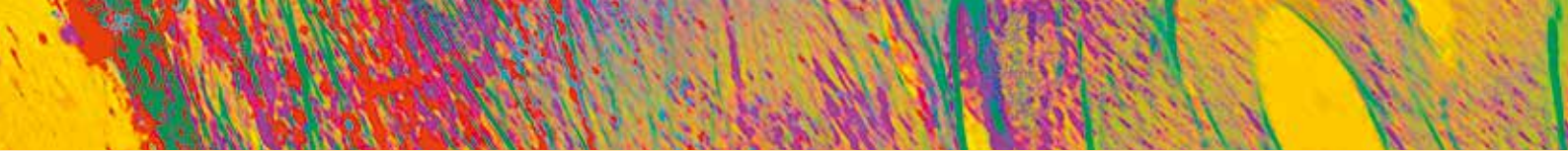
- High yield of pure and viable CD271<sup>+</sup> MSCs with CD271 (LNGFR) MicroBead Kit, human
- Isolation of MSCs with high proliferative potential, using highly specific marker Anti-MSCA-1 (W8B2) MicroBead Kit, human
- Fast and easy, 1 hour protocol

Automated isolation can be performed on the autoMACS® Pro Separator.

Products	Order no.
CD271 MicroBead Kit, human	130-099-023
Anti-MSCA-1 (W8B2) MicroBead Kit, human	130-093-583
CD271 MicroBead Kit (APC), human	130-092-283
CD271 MicroBead Kit (PE), human	130-092-819



Figure 1: CD271 MicroBead Kit, human



### 3. MSC expansion with StemMACS™ MSC Expansion Media Kit XF

- Robust expansion of human MSCs
- Xeno- and serum-free medium
- No substrate pre-coating of the culture vessels required
- No additional supplement, growth factors or serum required
- Preserves differentiation potential and immunomodulation ability

To support clinical translation, an upgrade to MACS® GMP medium is possible.

Products	Order no.
StemMACS MSC Expansion Media Kit XF, human	130-104-182



Figure 2: StemMACS MSC Expansion Media Kit XF

### 4. Cultured MSC phenotyping with MSC Phenotyping Kit

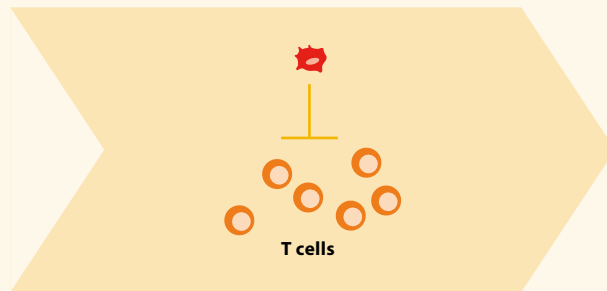
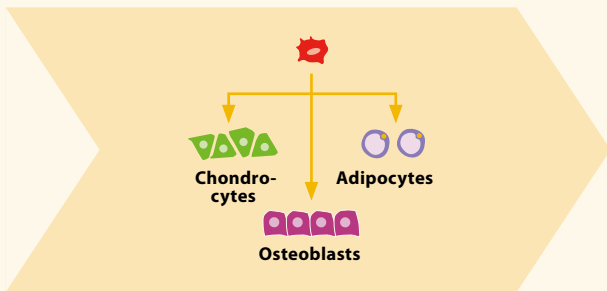
- Standardized identification and quantification of cultivated MSCs by flow cytometry
- Based on the defined ISCT standard
- 30 minutes protocol

**Kit includes:**

- A cocktail of seven fluorochrome-conjugated antibodies, including MSC characterization markers (CD73, CD90, CD105) and exclusion markers (CD14, CD20, CD34, and CD45)
- A cocktail of five fluorochrome-conjugated antibodies for isotype control
- Five single antibodies for compensation of PerCP, PE, APC and FITC channel

Automated and accurate cell count can be performed by MACSQuant® Flow Cytometers.

Products	Order no.
MSC Phenotyping Kit, human	130-095-198



## 5. MSC function analysis – differentiation with StemMACS™ AdipoDiff Media, StemMACS OsteoDiff Media and StemMACS ChondroDiff Media

- Supports differentiation of MSCs to adipocytes, osteoblasts, and chondrocytes
- Suitable for the analysis or quality control of the differentiation capacity of expanded MSCs
- Suitable for *in vitro* studies of processes involved in MSC differentiation, including gene expression and protein profiling

## 6. MSC function analysis – immuno-modulation with MSC Suppression Inspector Kit

- Standardized characterization of immuno-modulatory function of MSCs by co-culturing with CD4<sup>+</sup>CD25<sup>-</sup> or CD4<sup>+</sup> responder T cells (Tresp)
- Anti-Biotin MACSiBead™ Particles pre-loaded with biotinylated CD2, CD3, and CD28 antibodies for optimal T cell stimulation
- Detailed protocol includes Tresp isolation and stimulation, MSC suppression, and Tresp cell proliferation, determined by 3H-thymidine incorporation as well as carboxyfluorescein succinimidyl ester (CFSE) staining

Products	Order no.
StemMACS OsteoDiff, human	130-091-678
StemMACS AdipoDiff, human	130-091-677
StemMACS ChondroDiff, human	130-091-679

Products	Order no.
MSC Suppression Inspector, human	130-096-207
StemMACS MSC Expansion Media Kit XF, human	130-104-182
CD4 <sup>+</sup> CD25 <sup>+</sup> Regulatory T Cell Isolation Kit, human	130-091-301
CD4 <sup>+</sup> CD25 <sup>+</sup> CD127 <sup>dim/-</sup> Regulatory T Cell Isolation kit II, human	130-094-775



**Figure 3:** StemMACS AdipoDiff, StemMACS ChondroDiff, and StemMACS OsteoDiff

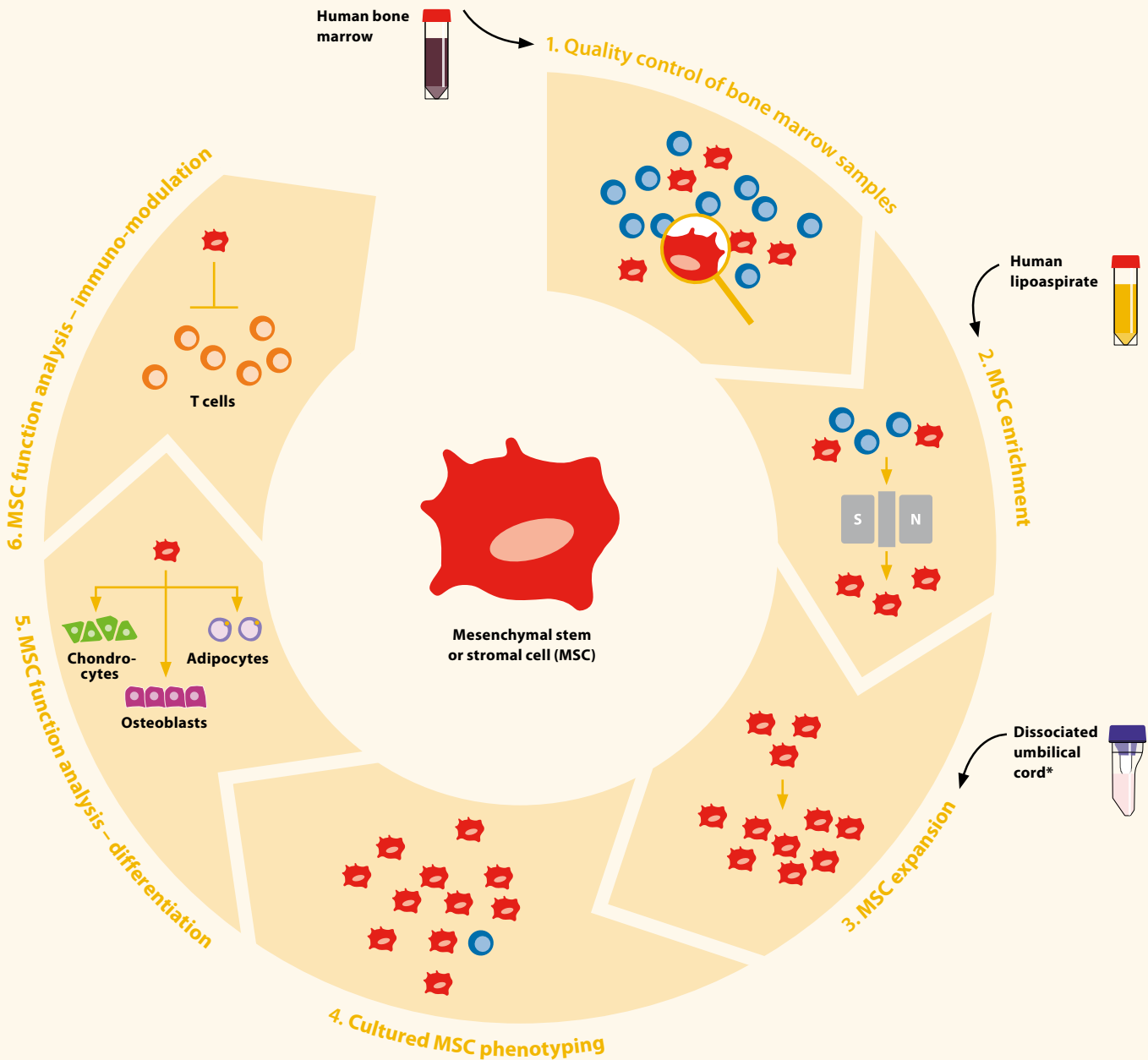


Learn more about the effective licensing of human mesenchymal stem cells. Visit [www.miltenyibiotec.com/MSClensing](http://www.miltenyibiotec.com/MSClensing)

# MSC workflow – Overview

Mesenchymal stem or stromal cells (MSCs) can be isolated from various tissues including bone marrow, adipose, umbilical cord, etc. Expanded MSCs, which possess the capacity for homing, immuno-modulation, promotion of repair, and direct regeneration of injured tissues, are promising therapeutic candidates for different diseases. Miltenyi Biotec is dedicated to develop products to enhance

MSC research and clinical applications. Here we show you the workflow overview for bone marrow-derived, as well as adipose tissue and umbilical cord-derived MSCs. This includes all steps from standardized MSC characterization and quantification, superior expansion in xeno- and serum-free medium, to MSC functional analysis like differentiation and immuno-modulation.



\* Use Umbilical Cord Dissociation Kit, human (Order no. 130-105-737) together with gentleMACS™ Dissociators.



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