

# Minimum criteria for adipose derived stem cells

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Adipose derived stem cells (ASCs) are derived from mesenchyme and have several general or unique characteristics. In order to be recognized as a mesenchymal stem cells, the following three criteria shall be satisfied. MSC must be plastic-adherent in standard culture conditions. The cells must be able to differentiate into trilineage mesenchymal differentiation pathways. ASCs must have a specific cell surface antigen expression.

Since ASCs are a kind of MSCs, they must meet the general conditions for being recognized as MSCs. In order to be recognized as a MSC, the following three criteria shall be satisfied.

Firstly, MSC must be plastic-adherent when maintained in standard culture conditions using tissue culture flasks [1]. While human bone marrow derived mesenchymal progenitor cells may be maintained, and possibly proliferated without attachment, these protocols require very specific culture conditions [2].

Secondly, the cells must be able to differentiate to adipocytes, osteoblasts and chondroblasts (trilineage mesenchymal differentiation) under standard differentiating culture conditions. Adipocyte differentiation is most readily demonstrated by staining with Oil Red O. Osteoblasts differentiation can be confirmed by staining with Alizarin Red or von Kossa staining. Chondroblast differentiation can be demonstrated by staining with Alcian blue or immunohistochemical staining for collagen type II [1].

Thirdly, the specific cell population can be identified by surface antigens expression. The surface antigens expression of MSCs include integrin-like adhesion molecules (CD29[beta-1 integrin, which plays a critical role in therapeutic angiogenesis]), hyaluronate receptors (CD44[crucial in the development of neoextracellular matrix]), CD 49e (alpha-5 integrin, important for cell adhesion to fibronectin]), extracellular matrix proteins (CD90, 105), intracellular adhesion molecules (CK54), vascular adhesion molecules (CK106), complement regulatory proteins, and histocompatibility antigens (Table 1) [3,4].

The mesenchymal and tissue stem cell committee of the international society of cellular therapy suggested expression of CD105, CD73, CD90, lack of expression of CD45, CD34, CD14 or CD11b, CD79a or CD19 and HLA-DR surface molecules as minimum criteria to define human MSCs [1]. In more detail, MSC, 95% of the MSC population must co-express MSC markers, CD105 (known as endoglin), CD73 (known as ecto- 5'- nucleotidase and CD90 (also known as Thy-1) as measured by flow cytometry [1].

Additionally, ASC should be devoid of hematopoietic antigen expression (lack of expression of CD45, CD34, CD14 or CD11b, CD79 a or CD19 and HLA-DR surface molecules). These cells must lack expression ( ≤ 2% positive) of CD45 (a pan-leukocyte marker),

CD34 (primitive hematopoietic progenitors and endothelial cells) CD14 or CD11b (CD14 and CD11b are prominently expressed on monocytes and macrophages, the most likely hematopoietic cells to be found in an MSC culture), CD79α or CD19 (markers of B cells that may also adhere to MSC in culture and remain vital through stromal interactions) and HLA class DR (molecules are not expressed on MSC unless stimulated, e.g. by IFN-γ). ASCs do not express HLA-DR protein and most MHC Class I molecules, suggesting that allogeneic transplantation is possible [4]. The cell surface phenotype of ASC is very similar to that of BM-MSC [5] (Table 2).

**Table 1.** Summary of surface phenotype criteria to identify MSC

Positive (≥95% )	Negative (≤2%)
CD105	CD45
CD73	CD34
CD90	CD14 or CD11b
	CD79α or CD19
	HLA-DR

**Table 2.** Comparison of cell surface antigens of ASC and BM-MSC

Surface marker	ASC	BM-MSC
CD9	+	+
CD10	+	+
CD13	+	+
CD29	+	+
CD31	-	-
CD34	-	-
CD44	+	+
CD45	-	-
<b>CD49d</b>	+	-
CD49e	+	+
CD54	+	+
CD55	+	+
CD59	+	+
CD90	+	+
CD105	+	+
<b>CD106</b>	-	+
CD117	+	+
CD146	+	+
CD166	+	+
STRO-1	+	+

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It is not routine MSC confirmation to verify normal karyotype to see if a chromosome aberration has been occurred [1]. These conditions should be applied only to human MSC and the antigen expression recommended may not apply to nonhuman systems [1].

In summary, in order to be recognized as a MSC, the three criteria (plastic adhesion, trilineage differentiating capacity, specific surface antigen expression) must be met. ASCs has a characteristic surface antigen expression that is slightly different from that of BM-MSC.

### Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflicts of interest.

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