

# Isolation and cultivation of adipose tissue-derived mesenchymal stromal cells

*Commonly used acronym: AT-MSC*

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## SCOPE OF THE METHOD

<b>The Method relates to</b>	Animal health
<b>The Method is situated in</b>	Basic Research
<b>Type of method</b>	In vitro - Ex vivo
<b>This method makes use of</b>	Human derived cells / tissues / organs
<b>Specify the type of cells/tissues/organs</b>	adipose tissue-derived mesenchymal stromal cells

## DESCRIPTION

### Method keywords

adipose tissue

Stem cells

mesenchymal stromal cells

isolation

cultivation

## **Scientific area keywords**

stem cell culture

stem cell isolation

mesenchymal stromal cells

## **Method description**

Approximately 125 g of processed adipose tissue is incubated for 90 minutes at 37°C in dissociation medium (1:1) consisting of 1% (v/v) bovine serum albumin and 1 mg/mL collagenase A in phosphate buffered saline (PBS). After two filtration steps, the filtrate is carefully brought on top of 15 mL of Histopaque®-1077. Upon centrifugation for 20 minutes at 1000 g (4°C), the top layer is removed and the AT-MSCs are collected in 50 mL PBS/BSA (1%). This procedure is carried out separately on two pieces of adipose tissue. Typically 5 - 20 x 10<sup>7</sup> viable cells are obtained per 250 g of processed adipose tissue. The isolated AT-MSCs are then (sub)cultured as a monolayer in AT-MSCs growth medium for 2 weeks, consisting of Dulbecco's Modified Eagle Medium supplemented with 10% (v/v) foetal bovine serum (FBS), 50 µg/mL streptomycin sulphate, 7.33 IU/mL benzyl penicillin and 2.5 µg/mL fungizone. Cell cultures are incubated at 37°C in a 5% (v/v) CO<sub>2</sub> humidified atmosphere and passaged at subconfluency using TrypLE® express. Growth media is changed every 3 days.

## **Lab equipment**

Biosafety cabinet level 2;

Cell incubator;

Centrifuge.

## **Method status**

History of use

Internally validated

Published in peer reviewed journal

## **PROS, CONS & FUTURE POTENTIAL**

### **Advantages**

Robust isolation method for adipose tissue-derived mesenchymal stromal cells.

## **REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION**

### **References**

De Kock J, Najar M, Bolleyn J, Al Battah F, Rodrigues RM, Buyl K, Raicevic G, Govaere O, Branson S, Meganathan K, Gaspar JA, Roskams T, Sachinidis A, Lagneaux L, Vanhaecke T, Rogiers V. (2012) Mesoderm-derived stem cells: the link between the transcriptome and their differentiation potential. *Stem Cells Dev.* 21(18):3309-23

Najar M, Rodrigues RM, Buyl K, Branson S, Vanhaecke T, Lagneaux L, Rogiers V, De Kock J. (2014) Proliferative and phenotypical characteristics of human adipose tissue-derived stem cells: comparison of Ficoll gradient centrifugation and red blood cell lysis buffer treatment purification methods. *Cytotherapy.* 16(9):1220-8

### **Associated documents**

## **PARTNERS AND COLLABORATIONS**

### **Organisation**

**Name of the organisation** Vrije Universiteit Brussel (VUB)

**Department** Pharmaceutical and Pharmacological Sciences

**Specific Research Group or Service** In Vitro Toxicology and Dermato-Cosmetology

**Country** Belgium

**Geographical Area** Brussels Region

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