

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

**Commonly used acronym:** AT-MSc

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### Contact person

Joery De Kock

### 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

## 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>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-MSC are collected in 50 mL PBS/BSA (1%). This procedure is carried out separately on two pieces of adipose tissue. Typically 5 - 20 x 10E7 viable cells are obtained per 250 g of processed adipose tissue. The isolated AT-MSC are then (sub)cultured as a monolayer in AT-MSC 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) CO2 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

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