

Laboratory Note

Isolation of Human Adipocytes with Collagenase NB 6 GMP Grade

1. Prepare on ice a solution of 0.45 PZ U/ml Collagenase NB 6 GMP Grade (100 mg: Cat. No. N0002880; 1 g: Cat. No. N0002779) in PBS (phosphate buffered saline solution).* Dissolve the collagenase on ice by adding small portions of lyophilisate at a time to avoid clumping. Collagenase NB 6 GMP Grade solution can be aliquoted and stored at -20 °C for 1 year.
2. Obtain liposuction tissue (e.g. 30 mL) and transfer it to 50 mL centrifuge tubes.
3. Centrifuge at 200 x g (rcf) for 5 minutes at room temperature. There will be three layers:
 - Top layer: adipose tissue
 - Second layer: tumescent waste
 - Third layer: stromal cell pellet
4. Take the top layer (adipose tissue) and transfer it to a new centrifuge tube (e.g. 5 mL tissue). Discard the second and third layer.
5. Dilute the adipose tissue by adding an equal volume of collagenase solution (e.g. 5 mL, see step 1) to each of the centrifuge tubes.
6. Incubate for 25 minutes at 37 °C while shaking (~ 54 rpm).
7. Centrifuge the digested adipose tissue at 200 x g (rcf) for 5 minutes at room temperature. There will be three layers:
 - Top layer: disintegrated tissue
 - Second layer: waste
 - Third layer: stromal vascular fraction (SVF) pellet
8. Dispose the first two layers without disturbing the SVF pellet.
9. To stop the enzymatic reaction resuspend the cells by adding medium (MEM - Modified Eagle Medium) with 10 % patient's own serum to the remaining third layer.
10. Perform a cell count. About 5×10^6 cells can be expected per mL of adipose tissue.

* e.g. 1 g Collagenase NB 6 GMP Grade in 310 mL PBS results in a concentration of 0.45 PZ U/mL if the lot has an activity of 0.14 PZ U/mg. The activity in PZ U/mg is stated on the Certificate of Analysis.

This protocol is supplied courtesy of Invitrx Therapeutics, Inc, USA. The user of this protocol is solely responsible and liable.

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Ordering

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