

# Preparation of Adipocytes (collagenase digestion)

## Materials:

- autoclaved forcipis, scissors
- sterile syringes, needles, scalpels, pincets
- sterile-filters for syringes
- 70µm cell-strainer (Falcon 2350)
- 40µm cell-strainer (Falcon 2340)
- HBSS modified (Sigma H-2387) containing antibiotics and 0.01M Hepes
- DMEM/F-12 (Sigma D-8900) containing antibiotics, 20% FCS (=Medium I)
- DMEM/F-12 (Sigma D-8900) containing antibiotics, 33µM Biotin (Sigma B-3639), 17µM Panthothenate (Sigma P-5155), 1nM T3 (Sigma T-6397), 100nM Dexamethasone (Sigma D-4902), 1µM Pioglitazone (Takeda), 500nM Insulin (Novo Nordisk "Actrapid®"), 250nM IBMX (Sigma I-7018) (=Medium II)
- DMEM/F-12 (Sigma D-8900) containing antibiotics, 33µM Biotin (Sigma B-3639), 17µM Panthothenate (Sigma P-5155), 1nM T3 (Sigma T-6397), 100nM Dexamethasone (Sigma D-4902), 1µM Pioglitazone (Takeda), 500nM Insulin (Novo Nordisk "Actrapid®"), 250nM IBMX (=Medium III)
- Collagenase solution (Collagenase Type IV (Sigma C-5138), 2mg/ml in HBSS modified), sterile filtered
- Red cell lysis buffer containing 0.154M NH<sub>4</sub>Cl, 10mM KHCO<sub>3</sub>, 0.1mM EDTA (=RCLB), sterile filtered
- Gelatine solution (1% in PBS)
- all solutions prewarmed to 37°C
- tissue culture plastic (centrifuge tubes, dishes, flasks, cell-scrappers, ...)

## Procedure:

1. Cut fat into small pieces (approx. 3x3mm)
2. Place pieces into 50ml tube containing 10-25ml Collagenase solution
3. Incubate for 60 minutes at 37°C in a shaking incubator
4. Filter solution through a 70µm cell-strainer into a new centrifuge tube
5. Centrifuge for 10 minutes at 200g
6. Resuspend pellet in 20ml Medium
7. Filter solution through a 40µm cell-strainer into a new centrifuge tube
8. Centrifuge for 5 minutes at 1500 rpm
9. Resuspend pellet in 20ml RCLB
10. Incubate for 10 minutes at room temperature
11. Centrifuge for 5 minutes at 1500 rpm
12. Resuspend pellet in 10-20ml Medium I
13. Transfer resuspended cells into an appropriate tissue culture vessel, coated with 1% Gelatine
14. Incubate at 37°C, 5% CO<sub>2</sub> for 24 hours
15. Replace containing medium with Medium II
16. Incubate at 37°C, 5% CO<sub>2</sub> for 48 hours
17. Replace one half of the containing medium with Medium II
18. Incubate at 37°C, 5% CO<sub>2</sub> for 48 hours
19. Replace one half of the containing medium with Medium III
20. Incubate at 37°C, 5% CO<sub>2</sub> for 48 hours
21. Replace one half of the containing medium with Medium III every 48 hours for 10 to 14 days until cells form lipid droplets

**Be careful to keep cells covered with medium during all medium replacements, otherwise lipid droplets will break. Do not tap or shake cells!**