**SURAT TUGAS**
Nomor: 1287-R/UNTAR/PENELITIAN/II/2023

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**SIUFUI HENDRAWAN, dr., M.Biomed., Dr.**

Untuk melaksanakan kegiatan penelitian/publikasi ilmiah dengan data sebagai berikut:

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| Judul | : | Rat Dermal Fibroblast Isolation And Culture |
| Nama Media | : | DJKI |
| Penerbit | : | DIREKTORAT JENDERAL KEKAYAAN INTELEKTUAL |
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Demikian Surat Tugas ini dibuat, untuk dilaksanakan dengan sebaik-baiknya dan melaporkan hasil penugasan tersebut kepada Rektor Universitas Tarumanagara

01 Februari 2023
**Rektor**

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# SURAT PENCATATAN
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| Nomor dan tanggal permohonan | : | EC00202269171, 27 September 2022 |
| **Pencipta** | | |
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| Jenis Ciptaan | : | **Buku Panduan/Petunjuk** |
| Judul Ciptaan | : | **Rat Dermal Fibroblast Isolation And Culture** |
| Tanggal dan tempat diumumkan untuk pertama kali di wilayah Indonesia atau di luar wilayah Indonesia | : | 27 September 2022, di DKI Jakarta |
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| | First draft: IAZ (German) |

## Materials:

1. Lab coat
2. Electric razor
3. Blade razor
4. Sterile surgery scissors, clamps and forceps
5. Sterile surgery tray and needles / tape
6. Sterile surgery drape
7. Sharp container
8. Sterile petri dish (2 ea)
9. Sponge and soap
10. Sterile small tea strainer (1 ea)
11. Sterile 250 mL beaker glass (1 ea)
12. Cell counting chamber with cover

## Solutions / Media:

1. Ketamine injection (Ilium, Troy laboratories pty limited Australia)
2. Xylazine (Ilium, Troy laboratories pty limited Australia)
3. Dulbeco's PBS solution without ca/mg (Sigma Cat # D8537 or Gibco cat #21600-010) containing 2% antibiotic/antimycotic (AB/AM) (Sigma Cat #A5955)
4. CASO agar (Fluka Cat #22095)
5. EGTA buffer solution containing 2% (AB/AM) (Sigma Cat #E4378)
6. 200 U/mL collagenase solution (freshly prepared from 2000 U/mL collagenase type I solution – see appendix) (Gibco Cat # 17100-017)
7. DMEM media culture (Sigma Cat #D5648 or Gibco Cat #11965) completed with 10-20% FBS (Gibco Cat #10270), 2% AB/AM, 2 mM L-glutamin (Sigma Cat #G7513), 1 mM Na-pyruvat (Sigma Cat #S8636) and 10 mM HEPES (Sigma Cat #H0887)
8. 0.36% and 0.22% Trypan Blue solution (Sigma cat# T8154)

## Consumables:

1. Nitrile gloves
2. Surgery mask
3. 1 mL syringe with needle
4. 70 % ethanol
5. 70 % ethanol spray
6. Sterile cotton balls
7. Small and big autoclavable bags
8. Used newspaper

9. Cell scraper
10. 100 um nylon cell sieve (Falcon, Durham, USA, Cat #352260)
11. 15 and 50 mL centrifuge tube
12. 2 ml, 5 ml, 10 ml and 25 ml serological pipet
13. 10 ml wide mouth serological pipet
14. T25 and T75 collagen-coated flasks (see appendix)

Notes:
Sigma, Saint Louis, USA
Gibco, Grand Island, NY, USA
Fluka, Sigma Aldrich Chemie Gmbh, Riedstr, Steinheim

## Work sheet:

Name of officer : _____
Rat type : _____
Rat's age : _____
Date of surgery : _____

sticker

**Researcher :**

**A. Biopsy of rat skin**

☐ Weigh and anesthetize rat with mix of 150 ul ketamine and 50 ul xylazine for 100-200 mg of body weight, wait until the rat fall asleep
Rat's weight: _____ gr

☐ Shave rat's hair on stomach/abdomen area with electric razor

☐ Clean/wipe the hair with sponge wetted with soap

☐ Continue shaving with blade razor to remove all the fine hair. If the hair/abdomen area become dry before all the hair has gone, again wipe the area with soap-wetted sponge

☐ Clean rat's hair debris by wiping the shaved area with 70% ethanol-wetted cotton

☐ Cut the desired size of rat skin off covering the dermis part

☐ Put skin on sterile petri dish, add _____ ml (target: 20-30 ml) pre-warmed PBS containing 2% antibiotic/antimycotic (AB/AM) until covering all skin surface and transport to the lab

**Researcher :**

**B. Sterility test in laminar flow:**

☐ Pipette _____ ml (target: 0.3-0.5 ml) of transport solution to a CASO-agar plate, let it diffuse into agar, seal with parafilm, and incubate at 32 °C (upside-down).

☐ Put the skin tissue with sterile forceps to the agar surface of a 2nd CASO-agar plate (weight and roll it over), seal with parafilm and incubate at 32 °C (upside-down).
Weight & size of the skin tissue: _____ g & _____cm x _____cm
Macroscopic evaluation:

_____
_____
_____

**Researcher :**

**C. Fibroblast Isolation**

☐ Transfer the skin into a new petri dish

☐ Immediately add _____ ml (target: 20-30 ml) pre-warmed PBS with 2% AB/AM.

☐ Shake skin very gently to wash/remove all debris and excess hair.

☐ Aspirate and discard the solution from petri dish

☐ Repeat this PBS washing step 5 times

☐ Change of equipment: new sterile Petri dish and forceps

☐ Add pre-warmed wash/EGTA solution containing 2% AB/AM until covering all skin surface _____ ml (target: 20-30 ml)

☐ Shake the skin very gently to wash/remove all debris and excess hair

☐ Discard the solution from petri dish

☐ Repeat this EGTA washing step 5 times

☐ Change of equipment: new sterile Petri dish and forceps

☐ Cut the skin into two pieces of 2 x 1 cm size

☐ Add freshly-prepared 200 Units/ml collagenase in complete DMEM media containing 10-20% FBS, 2% AB/AM, 2mM L-glutamine and 1 mM Na-pyruvate solution (See appendix 1 for collagenase solution preparation) until covering all skin surface _____ ml (target: 20-30 ml)

☐ With the dermis-side down, incubate the skin at 37C, 5% CO2 for 3-5 hours

☐ Observe occasionally whether there are cells released into the solution

☐ After 3-5 h incubation, scrap the dermis side with cell scraper gently until all dermis part dissolves into collagenase solution

☐ Discard the epidermis part

☐ Pipette the cell solution with wide-mouth serological pipette and scrap gently onto tea strainer sieve. Rinse thoroughly with _____ ml PBS (2% AB/AM)

☐ Pipette and sieve cell suspension with 100 um nylon cell sieve, rinse thoroughly with _____ ml PBS (2% AB/AM)

☐ Collect cells into a 50 ml centrifuge tube

☐ Centrifuge 130 g for 10 minutes

☐ Discard supernatant, re-suspend cells with 7 ml complete DMEM media containing 10-20% FBS, 2% AB/AM, 2 mM L-glutamine, 1mM Na-pyruvate and 10 mM HEPES

☐ Collect cells into a 50 ml centrifuge tube

☐ Discard supernatant, re-suspend cells with 4 ml complete DMEM media

☐ Take a small amount (target: 50-100 µl) suspension into 1.5 ml microtubes and count the cells in improved chamber with trypan blue staining.

☐ Mark the flask with primary fibroblasts of rat skin, name of operator, date of seeding

☐ Incubate at 37ºC, 5% CO2 for 30 min

☐ After 30 min incubation, fibroblast cells will attach on collagen-coated surface of the flask whereas the non-fibroblast cells remain flying (non-attached) in the culture media

☐ Collect the media from flask into 15 ml centrifuge tube and keep it for the media leftover cell counting

☐ Add new complete DMEM media _____ ml (target: 4-5 ml for T25 flask)

☐ Incubate at 37C, 5% $CO_2$ for certain/desired time or until flask surface is 80% covered by cells with media change every one or two days

**D. Cells counting in a Neubauer-Tuerk improved chamber:**

☐ Mix trypan blue and cell suspension dilution: ____ ul Tr.B + ____ ul cell suspension (1: ___ )

☐ Leave the Tr.B-cell suspension for 2 mins before counting

☐ PBS re-dilution: ____ ul Tr.B & cell susp mix + ____ ul PBS* (1: ___ )

☐ Use trypan blue 0.22% for 10x dilution and 0.36% for 2x dilution. If debris is too much that can interfere the counting, dilute for the second time the Tr.Blue-cells mixture with PBS into higher dilution.

☐ **Counting formula**

***Cells/ml** = (viable + dead) cells/square x dilution factor x common factor (Improved Neubauer:$10^4$)

***Total cells** = cells / ml x total volume

***Viability** = viable cells / (viable + dead) cells x 100%

☐ **Isolated cells (IC) counting**

**Counter I** : ☐☐

Total volume of cells suspension : _____ ml
Dilution factor : _____
Viable cells : _____/_____ squares
Dead cells : _____/_____ squares
Viable + dead cells : _____/_____ squares

Fibroblast cells per ml : _____
Total fibroblast cells : _____
Viability : _____%

**Counter II** : ☐☐

Total volume of cells suspension : _____ ml
Dilution factor : _____
Viable cells : _____/_____ squares
Dead cells : _____/_____ squares
Viable + dead cells : _____/_____ squares

Fibroblast cells per ml : _____
Total fibroblast cells : _____
Viability : _____%

**Resume**
**Fibroblast cells/ml** : _____
**Total fibroblast cells** : _____
**Viability** : _____ **%**

☐ **Cells in Leftover Media (CILOM) counting**

**Counter I** : ☐☐

Total volume of cells suspension : _____ ml
Dilution factor : _____
Viable cells : _____/_____ squares
Dead cells : _____/_____ squares
Viable + dead cells : _____/_____ squares

Fibroblast cells per ml : _____
Total fibroblast cells : _____
Viability : _____%

**Counter II** : ☐☐

Total volume of cells suspension : _____ ml
Dilution factor : _____
Viable cells : _____/_____ squares
Dead cells : _____/_____ squares
Viable + dead cells : _____/_____ squares

Fibroblast cells per ml : _____
Total fibroblast cells : _____
Viability : _____%

**Resume**
**Fibroblast cells/ml** : _____
**Total fibroblast cells** : _____
**Viability** : _____ **%**

**Adherence calculation:**
Total cells seeded (IC) : _____
Total cells in CILOM : _____

Adhered cells : _____

**Adherence of cells:**
adhered cells / seeded cells X 100% = _____ %

## Sterility tests

| CASO Agar | Bacterial growth after 3 days |
|---|---|
| Rat skin | ☐ Yes ☐ No |
| Medium transport rat skin | ☐ Yes ☐ No |

**Researcher :**

**E. Trypsinizing and culturing fibroblast**

☐ Discard DMEM media from T25 flask

☐ Add _____ ml (target: 1 ml / 25 $cm^2$ flask) of 0.05% trypsin-EDTA or until all cells are covered enough. Spread thoroughly over cells' surface

☐ Incubate in maximum 15 mins at 37ºC with 3-5 mins interval

☐ Gently tab flask to dislodge the cells

☐ Add _____ ml (target: 2-4 ml / double volume of trypsin) complete DMEM media, mix gently and thoroughly to stop / inactivate trypsin

☐ Collect cells suspension in tube and centrifuge at 130 g for 10 mins

☐ Discard supernatant and re-suspend cells in 4 ml complete DMEM media

☐ Take a small amount (target: 50-100 µl) suspension into 1.5 ml microtubes and count the cells in improved chamber with tryphan blue staining.

☐ Transfer cells into T75 collagen-coated flask (or other bigger size flask) and add media up to the allowed volume for the flask _____ ml (target : 12-15 ml for T75)

☐ Mark the flask with number of cells, name of cells, name of operator, date of surgery, number and date of passage, and name of culture media

☐ **Isolated fibroblast cells (IFC) counting**

**Counter I** : ☐☐

Total volume of cells suspension : _____ ml
Dilution factor : _____
Viable cells : _____/_____ squares
Dead cells : _____/_____ squares
Viable + dead cells : _____/_____ squares

Fibroblast cells per ml : _____
Total fibroblast cells : _____
Viability : _____%

**Counter II** : ☐☐

Total volume of cells suspension : _____ ml
Dilution factor : _____
Viable cells : _____/_____ squares
Dead cells : _____/_____ squares
Viable + dead cells : _____/_____ squares

Fibroblast cells per ml : _____
Total fibroblast cells : _____
Viability : _____%

**Resume**
**Fibroblast cells/ml : _____**
**Total fibroblast cells : _____**
**Viability : _____ %**

**Researcher :**

**F. Fibroblast function analysis**

- Collagen amount produced from fibroblasts is assessed to evaluate the cell functionality
- Collagen production is determined with Sirius Red Total Collagen Detection Assay Kit (Chondrex Cat No#9062)
- Cell culture media (supernatants) are collected after the cells are confluent and analysed according to the manufacturer's protocol

| Validation | Prepared by: | Checked by: | Authorized by: |
|---|---|---|---|
| Name | Jennifer Lheman | dr. Siufui Hendrawan | Prof. Dr. med. Hans U Baer |
| Signature | | | |
| Date | 27 September 2022 | | |

**APPENDIX :**

## 1. 2000 U/ml Collagenase Stock Solution Preparation

- ☐ Weigh _____ mg lyophilized collagenase (Gibco cat # 17100-017) to get 2000 U/ml (**depends on Unit concentration of each batch**) to get 2000 U/ml final concentration in _____ mL enzyme buffer.
- ☐ Dissolve collagenase in enzyme buffer and sterile filter it with 0,22 μm pore size.
- ☐ Before use, dilute the 2000 U/mL collagenase solution into 200 U/mL final concentration with complete media culture (complete DMEM media)

## 2. Coating culture flask with collagen

- ☐ Pipette _____ mL (until all flask's bottom surface is covered) collagen solution into flask
- ☐ Shake the flask gently to spread the solution thoroughly
- ☐ Pipette out all collagen solution, re-cap flask and let dry for couple days
- ☐ Pipette _____ mL (until all flask's bottom surface is covered) PBS solution into flask
- ☐ Shake the flask gently to spread the solution thoroughly
- ☐ Pipette out all PBS solution, re-cap flask and let dry for couple days