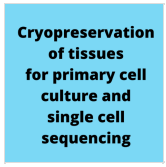

Jul 01, 2020

Cryopreservation of tissues for primary cell culture and single cell sequencing

Harikrishna Nakshatri[1]

[1]Indiana University/Purdue University at Indianapolis

1 Works for me dx.doi.org/10.17504/protocols.io.bhvjj64n

Human Cell Atlas Method Development Community

Harikrishna Nakshatri

ABSTRACT

This protocol details the cryopreservation of tissues used for primary cell cultures and single cell sequencing.

ATTACHMENTS

Cryopreservation_of_tissues_for_primary_cell_culture_and_single_cell_sequencing.docx

DOI

dx.doi.org/10.17504/protocols.io.bhvjj64n

PROTOCOL CITATION

Harikrishna Nakshatri 2020. Cryopreservation of tissues for primary cell culture and single cell sequencing. **protocols.io** dx.doi.org/10.17504/protocols.io.bhvjj64n

KEYWORDS

Cryopreservation, Primary Cell Culture, Single Cell Sequencing

CREATED

Jun 24, 2020

LAST MODIFIED

Jul 01, 2020

OWNERSHIP HISTORY

Jun 24, 2020 Megan Freund

Jul 01, 2020 Harikrishna Nakshatri

PROTOCOL INTEGER ID

38539

GUIDELINES

After you're done:

Thawing for single cell analyses or preparing for cells:

Unlike cooling, thawing has to be rapid at **37 °C** and transfer the content to **10 mL** warm media to wash tissues. Since DMSO is known to cause differentiation of stem cells, we wash tissues thoroughly before starting digestion (particularly for generating primary cell line).

MATERIALS TEXT

PRIMARY CELL F12-DMEM (low glucose) 3:1 Media

1) F12 (Cat# 11765-054, Gibco) ..... **375 ml**
2) DMEM (low glucose, Cat# 12320-032, Gibco) ..... **125 ml**
3) FBS (Cat# 26140-079, Gibco) ..... **25 ml**
4) Hydrocortisone (Cat# H0888, Sigma-Aldrich, 0.4 µg/ml). The stock is 1mg/ml **200 µl**
5) Penicillin-Streptomycin Solution, 100X (Cat# 30-002-CI, Corning) ..... **5 ml**
6) Insulin (Cat# I5500, Sigma-Aldrich, 5 µg/ml). The stock is 1 mg/ml ..... **2.5 ml**
7) EGF (Cat# 236-EG-200, R&D systems, 20 ng/ml). The stock is 2 µg/µl..... **5 µl**

Note: To the all cells, also add the following during culturing and changing the media. For **10 ml media** , use **40 µl of 6 mg/ml Adenine** and **5 µl of 10 mM ROCK inhibitor Y-27632** .

1) Adenine (Cat# A-9001, Sigma-Aldrich). The stock is 6mg/ml ..... **40 µl**
2) ROCK inhibitor (Y-27632, Cat# ALX-270-333-M005, Enzo Life Sciences). The stock is 10 mM ..... **5 µl**

Other Materials:

- Cryoprotective Freezing Medium (Lonza cat. no. 12-132A)
- CoolCell containers (Nalgene Cat#5100-0001)

SAFETY WARNINGS

Please see the Safety Data Sheet (SDS) for any protocol hazards and warnings.

DISCLAIMER:

Freezing Protocol

1 Collect tissues in the Cryoprotective Freezing Medium with ROCK inhibitor.

2 Mince tissues into small pieces.

3 Resuspend in **500 µl primary cell medium** and **500 µl cryoprotective freezing medium** +

Citation: Harikrishna Nakshatri (07/01/2020). Cryopreservation of tissues for primary cell culture and single cell sequencing. https://dx.doi.org/10.17504/protocols.io.bhvjj64n

0.5 µl ROCK inhibitor .

4 

Aliquot into cryogenic storage vials.

5 Place vials in CoolCell containers.

6 

Cells should be frozen slowly at **1 °C per minute** .

This can be achieved using the CoolCell containers at a **-70 °C** to **-90 °C** freezer overnight, then transferring to liquid nitrogen storage.

Citation: Harikrishna Nakshatri (07/01/2020). Cryopreservation of tissues for primary cell culture and single cell sequencing. https://dx.doi.org/10.17504/protocols.io.bhvjj64n