

**Cryopreservation of tissues for primary cell culture and single cell sequencing**

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# Cryopreservation of tissues for primary cell culture and single cell sequencing

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Human Cell Atlas Method Development Community

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## ABSTRACT

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

## ATTACHMENTS

Cryopreservation\_of\_tissue  
s\_for\_primary\_cell\_culture\_  
and\_single\_cell\_sequencin  
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## DOI

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## PROTOCOL CITATION

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## KEYWORDS

Cryopreservation, Primary Cell Culture, Single Cell Sequencing

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
## 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:

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#### 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** +

 **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.