

# Cryopreservation of mucosal biopsies

Sean M. Hughes, April L. Ferre, Sarah E. Yandura, Cory Shetler, Chris A. R. Baker, Fernanda Calienes, Claire N. Levy, Rena D. Astronomo, Zhiquan Shu, Gretchen M. Lentz, Michael Fialkow, Anna C. Kirby, M. Juliana McElrath, Elizabeth Sinclair, Lisa C. Rohan, Peter L. Anderson, Barbara L. Shacklett, Charlene S. Dezzutti, Dayong Gao, Florian Hladik

## Abstract

**Citation:** Sean M. Hughes, April L. Ferre, Sarah E. Yandura, Cory Shetler, Chris A. R. Baker, Fernanda Calienes, Claire N. Levy, Rena D. Astronomo, Zhiquan Shu, Gretchen M. Lentz, Michael Fialkow, Anna C. Kirby, M. Juliana McElrath, Elizabeth Sinclair, Lisa C. Rohan, Peter L. Anderson, Barbara L. Shacklett, Charlene S. Dezzutti, Dayong Gao, Florian Hladik  
Cryopreservation of mucosal biopsies. **protocols.io**

dx.doi.org/10.17504/protocols.io.p5adq2e

**Published:** 30 May 2018

## Materials

🧪 dimethylsulfoxide (DMSO) by [Sigma Aldrich](#)

✓ Fetal bovine serum by Contributed by users

🧪 Cryovials V7884 by [Millipore Sigma](#)

## Protocol

### Freezing protocol

#### Step 1.

Prepare cryopreservation medium (10% dimethylsulfoxide in fetal bovine serum).

🧴 **AMOUNT**

0.9 ml : fetal bovine serum

🧴 **AMOUNT**

0.1 ml : dimethylsulfoxide

#### Step 2.

Chill cryopreservation medium at 4C for at least 30 minutes.

🕒 **DURATION**

00:30:00 :

#### Step 3.

Aliquot 0.2 mL cryopreservation medium into each cryovial.

📌 **NOTES**

Larger volumes may be used with multiple biopsies. E.g. use 1 mL with 5-10 biopsies.

#### **Step 4.**

Place one or more biopsies in each cryovial.

#### **NOTES**

Ensure that the biopsies are completely covered with cryopreservation medium. Add more to cover if necessary.

#### **Step 5.**

Close cryovial, place in a Mr. Frosty, and freeze to -80C overnight.

#### **NOTES**

Any controlled rate cooling device that yields a decrease in temperature of 1C per minute can be used in place of a Mr. Frosty.

#### **Step 6.**

For storage, place samples in a liquid nitrogen freezer until needed.

#### Thawing protocol

#### **Step 7.**

Put 5mL of cell culture medium of interest into a plate or well.

#### **Step 8.**

Remove the cryovials from the liquid nitrogen freezer, but keep them on liquid nitrogen in a pan or other device for carrying liquid nitrogen until ready to thaw.

#### **Step 9.**

Transfer cryovials to a 37C water bath and agitate until thawed.

#### **Step 10.**

Transfer biopsies with forceps into 5 mL of room temperature culture medium.

#### **Step 11.**

Incubate for 10 minutes at room temperature.

#### **DURATION**

00:10:00 :

#### **Step 12.**

Use biopsies as needed.