Cryopreservation of mucosal biopsies

**PLOS One**


¹University of Washington

**Work for me** dx.doi.org/10.17504/protocols.io.p5adq2e

**EXTERNAL LINK**

https://doi.org/10.1371/journal.pone.0200653

**THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION**


**PROTOCOL CITATION**


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

**MANUSCRIPT CITATION**


**KEYWORDS**
cryopreservation, mucosa, colon, rectum, vagina, cervix

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**CREATED**

May 14, 2018
Freezing protocol

1. Prepare cryopreservation medium (10% dimethylsulfoxide in fetal bovine serum).
   - **0.9 mL** fetal bovine serum
   - **0.1 mL** dimethylsulfoxide

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

3. Aliquot 0.2 mL cryopreservation medium into each cryovial.
   - Larger volumes may be used with multiple biopsies. E.g. use 1 mL with 5-10 biopsies.

4. Place one or more biopsies in each cryovial.
   - Ensure that the biopsies are completely covered with cryopreservation medium. Add more to cover if necessary.

5. Close cryovial, place in a Mr. Frosty, and freeze to -80°C overnight.
   - Any controlled rate cooling device that yields a decrease in temperature of 1°C per minute can be used in place of a Mr. Frosty.

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

Thawing protocol

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

   Remove the cryovials from the liquid nitrogen freezer, but keep them on liquid nitrogen in a pan or other device for


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8  carrying liquid nitrogen until ready to thaw.

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

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

11 Incubate for 10 minutes at room temperature.

12 Use biopsies as needed.