JUL 31, 2018

OPEN ACCESS

DOI:
[dx.doi.org/10.17504/protocols.io.p6adrae](https://doi.org/10.17504/protocols.io.p6adrae)

External link:
<https://doi.org/10.1371/journal.pone.0200653>

Protocol 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 2018. Vitrification of mucosal biopsies. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.p6adrae>

MANUSCRIPT CITATION: Hughes SM, Ferre AL, Yandura SE, Shetler C, Baker CAR, Calienes F, Levy CN, Astronomo RD, Shu Z, Lentz GM, Fialkow M, Kirby AC, McElrath MJ, Sinclair E, Rohan LC, Anderson PL, Shacklett BL, Dezzutti CS, Gao D, Hladik F (2018) Cryopreservation of human mucosal tissues. PLoS ONE 13(7): e0200653. doi: [10.1371/journal.pone.0200653](https://doi.org/10.1371/journal.pone.0200653)

Vitrification of mucosal biopsies

PLOS One

April L.

Sean M. Hughes¹, Ferre,
 Chris A. R.
 Baker,

Sarah E. Yandura, Cory Shetler,
 Claire N.
 Fernanda Calienes, Levy,
 Gretchen M.

Rena D. Astronomo, Zhiquan Shu, Lentz,

Anna C.

M. Juliana

Michael Fialkow, Kirby,

McElrath,

Elizabeth

Lisa C.

Peter L.

Sinclair,

Rohan,

Anderson,

Barbara L.

Shacklett,

Charlene S. Dezzutti, Dayong Gao,

Florian Hladik¹

¹University of Washington

Sean Hughes

MATERIALS

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- Cryovials Millipore Sigma Catalog #V7884
- Ethylene glycol Contributed by users
- Dimethylsulfoxide Contributed by users
- Fetal bovine serum Contributed by users
- Phosphate-buffered saline, pH 7.4 Contributed by users
- Aluminum foil Contributed by users
- Liquid nitrogen Contributed by users

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Protocol status: Working

Created: May 15, 2018

Last Modified: Jul 31, 2018

PROTOCOL integer ID:
12194

Keywords: vitrification,
mucosa, colon, rectum,
vagina, cervix

Freezing procedure

- 1 Prepare explants/biopsies 5mmx5mm or smaller.
- 2 Prepare 1X vitrification medium (20% ethylene glycol, 20% dimethylsulfoxide in saline with fetal bovine serum).
  - 1 mL ethylene glycol
  - 1 mL dimethylsulfoxide
  - 0.6 mL fetal bovine serum
  - 2.4 mL phosphate-buffered saline
- 3 Prepare 0.5X vitrification medium (10% ethylene glycol, 10% dimethylsulfoxide in saline with fetal bovine serum).
  - 0.5 mL ethylene glycol

0.5 mL
dimethylsulfoxide

0.8 mL fetal bovine
serum

3.2 mL phosphate-buffered
saline

- 4 Prepare aluminum foil pieces. Cut rectangular pieces of foil that are just narrower than the width of a cryovial and just shorter than the height of the cryovial up to the threads of the cap.

Note

Check to see that each foil piece goes easily into a cryovial without catching.

- 5 Place 5 mL of the 0.5X vitrification medium in a well of a six-well plate. Place 5 mL of the 1X vitrification medium in a second well of a six-well plate.

- 6 Refrigerate the six-well plate at 4C for 30 min.

00:30:00

- 7 Prepare a pan of liquid nitrogen with absorbent cloth and place a cryovial rack so the bottom of the cryovials will be immersed in liquid nitrogen. Make sure that there is a part of the pan where enough liquid nitrogen is exposed that you can immerse the biopsies at least one inch into the liquid.

- 8 Place empty cryovials in a rack in the liquid nitrogen container so they are cold when you put the biopsy in.

- 9 Remove the six-well plate from the refrigerator and place in a biosafety cabinet.

- 10 Transfer biopsies with forceps into the 0.5X solution and incubate at room temperature for 5 minutes.

⌚ 00:05:00

- 11 Transfer biopsies with forceps into the 1X solution and incubate at room temperature for 5 minutes.
⌚ 00:05:00
- 12 After 5 minutes, briefly blot biopsies individually on a kimwipe or sterile wipe. This is to remove the vitrification medium that is coating the biopsies.
- 13 Place a biopsy close to the edge at the narrow end of a pre-cut piece of aluminum foil.
- 14 Pick up the other end of the aluminum foil with forceps.
- 15 Plunge the entire foil (and some of the forceps) into liquid nitrogen.
- 16 After about 10 seconds, when the bubbling has subsided, place the foil with the biopsy frozen to it into a cryovial (precooled in liquid nitrogen).

Note

3-4 biopsies can typically fit into one cryovial. Foil could be cut differently to allow more to fit.
- 17 Cap the cryovials and store in a liquid nitrogen freezer until needed.

Thawing procedure

- 18** Prepare thawing medium (10% ethylene glycol, 10% dimethylsulfoxide in culture medium of interest).

0.5 mL ethylene glycol

0.5 mL dimethylsulfoxide

4 mL cell culture medium

- 19** Place 5 mL of the thawing medium in a well of a six-well plate. Place 5 mL of plain culture medium into a second well and keep the plate at room temperature.

- 20** Remove the cryovials from the liquid nitrogen freezer, but keep them on liquid nitrogen in a pan or other device for carrying liquid nitrogen.

- 21** While keeping the cryovials on liquid nitrogen, unscrew the caps.

- 22** Use forceps to remove one piece of aluminum foil/biopsy from a cryovial.

- 23** Quickly move the foil out of the liquid nitrogen pan and place it, biopsy side down, in the well with the thawing medium and shake until biopsy detaches (~5 seconds).

Note

Several samples from the same donor can be thawed and transferred at the same time (a minute more or less of time in the thawing medium or culture medium doesn't make a difference).

- 24** Incubate at room temperature for 10 minutes.

00:10:00

25 Use forceps to transfer the biopsy to the culture medium.

26 Incubate at room temperature for 10 minutes.

27 The biopsy is ready for use.