Vitrification 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

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Materials

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

Protocol

Freezing procedure

Step 1.

Prepare explants/biopsies 5mmx5mm or smaller.

Step 2.

Prepare 1X vitrification medium (20% ethylene glycol, 20% dimethylsulfoxide in saline with fetal bovine serum).

AMOUNT
1 ml : ethylene glycol
AMOUNT
1 ml : dimethylsulfoxide
AMOUNT
0.6 ml : fetal bovine serum
AMOUNT
2.4 ml : phosphate-buffered saline

Step 3.

Prepare 0.5X vitrification medium (10% ethylene glycol, 10% dimethylsulfoxide in saline with fetal bovine serum).

AMOUNT
0.5 ml : ethylene glycol
AMOUNT
0.5 ml : dimethylsulfoxide
AMOUNT
0.8 ml : fetal bovine serum
AMOUNT
3.2 ml : phosphate-buffered saline

Step 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.

NOTES

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

Step 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.

Step 6.

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

DURATION
00:30:00 :

Step 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.

Step 8.

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

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

Step 10.

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

DURATION

00:05:00 :

Step 11.

Transfer biopsies with forceps into the 1X solution and incubate at room temperature for 5 minutes.

DURATION
00:05:00 :

Step 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.

Step 13.

Place a biopsy close to the edge at the narrow end of a pre-cut piece of aluminum foil.

Step 14.

Pick up the other end of the aluminum foil with forceps.

Step 15.

Plunge the entire foil (and some of the forceps) into liquid nitrogen.

Step 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).

NOTES

3-4 biopsies can typically fit into one cryovial. Foil could be cut differently to allow more to fit.

Step 17.

Cap the cryovials and store in a liquid nitrogen freezer until needed.

Thawing procedure

Step 18.

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

AMOUNT
0.5 ml : ethylene glycol

AMOUNT
0.5 ml : dimethylsulfoxide

AMOUNT
4 ml : cell culture medium

Step 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.

Step 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.

Step 21.

While keeping the cryovials on liquid nitrogen, unscrew the caps.

Step 22.

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

Step 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).

NOTES

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).

Step 24.

Incubate at room temperature for 10 minutes.

DURATION
00:10:00 :

Step 25.

Use forceps to transfer the biopsy to the culture medium.

Step 26.

Incubate at room temperature for 10 minutes.

DURATION
00:10:00 :

Step 27.

The biopsy is ready for use.