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Cryopreservation of mucosal biopsies

PLOS One

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MATERIALS

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⊗ dimethylsulfoxide (DMSO) **Sigma Aldrich**

⊗ Fetal bovine serum **Contributed by users**

⊗ Cryovials **Millipore Sigma Catalog #V7884**

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12162

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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 4C for at least 30 minutes.

🕒 00:30:00

- 3 Aliquot 0.2 mL cryopreservation medium into each cryovial.

Note

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.

Note

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 -80C overnight.

Note

Any controlled rate cooling device that yields a decrease in temperature of 1C 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.
- 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.
- 9 Transfer cryovials to a 37C 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.
00:10:00
- 12 Use biopsies as needed.