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Tissue freezing in Cryostor solution + processing

Regina Hoo[1], Carmen Sancho[1], Roser Vento-Tormo[2]
[1]Sanger Institute; [2]Wellcome Sanger Institute

1 Works for me dx.doi.org/10.17504/protocols.io.bgsnjwde

Vento-Tormo
Tech. support email: rv4@sanger.ac.uk

Regina Hoo

ABSTRACT

Tissue freezing in cryostor solution and recovery for tissue processing

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MATERIALS TEXT

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FBS **Invitrogen - Thermo Fisher**

RPMI 1640 Medium **Thermo Fisher Scientific Catalog #11875093**

Cryostar CS10 **Sigma Aldrich Catalog #C2874**

1 Chill Cryostor solution (CS10) on ice (C2874-Sigma).

2 Most tissue is collected in Hyperthermosol and kept on ice or at **4 °C** .

3 Place tissue in petri dish, add enough ice cold RPMI/10% FBS to cover the tissue (depend on tissue size).

4 Mince tissue with 2 scapels, just like what we would do for enzymatic digestion.

5 Carefully transfer minced tissue into a 50ml falcon tube.

6 Wash and rinse the petri dish with plenty of RPMI/10% FBS.
Transfer the RPMI/10%FBS and remaining tissue pieces into the falcon tube.

7 Centrifuge at 1500 rpm for 3 minutes.

8 Discard supernatant.

9 Resuspend the tissue in 1 ml of ice cod CS10 and transfer into a cryovial.

10 Rinse the falcon tube with 0.8 ml of extra cold CS10 and transfer into the cryovial.

11 Place cryovial in CoolCell and leave in **-80 °C** freezer overnight.

12 Remove cryovial from CoolCell next day, and store cryovial in designated box in **-80 °C** .

13 To process cryopreserved tissue, thaw cryovials in water bath at **37 °C** .

14 Transfer thawed tissue into a falcon tube containing 20ml warm RPMI/10% FBS.

15 Centrifuge at 500 x g for 4 minutes.

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16 Discard supernant. Proceed with preferred tissue digestion protocol

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