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Tissue freezing in Cryostor solution + processing

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ABSTRACT

Tissue freezing in cryostor solution and recovery for tissue processing

MATERIALS

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⊗ FBS Invitrogen - Thermo Fisher

⊗ RPMI 1640 Medium Thermo Fisher Scientific Catalog #11875093

⊗ Cryostar CS10 Sigma Aldrich Catalog #C2874

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Protocol status: Working
We use this protocol and it's working

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PROTOCOL integer ID:
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- 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 cold 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.
- 16 Discard supernant. Proceed with preferred tissue digestion protocol