HubMap UF TMC Tissue Dissociation to Single Cell

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GUIDELINES
Perform all steps using appropriate aseptic technique.

MATERIALS TEXT
MATERIALS

![Forceps Gold](https://biotechnology.com/catalog/f3001)

![Scissors Carl](https://roth.com/catalog/hct7.1)

![DMEM Gibco - Thermo](https://fischer.com/catalog/11885)

![Sterile deionized H2O Contributed by users](https://stemcell.com/catalog/10x)

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SAFETY WARNINGS

Use universal safety precautions when handling human samples and employ personal protective equipment (e.g.,

**Miltenyi C Tube** Contributed by users
**Sterile Petri Dishes** Contributed by users
**Disposable Scalpels 10 Blade** Contributed by users
**FBS USDA Origin** Contributed by users Catalog #25-550
**Penicillin/Streptomycin** Corning Catalog #30-002-CL
**0.5M EDTA** Boston Bioproducts Catalog #BM-150
**Ammonium Chloride** Fisher Scientific Catalog #A661-500
**NaHCO** Fisher Scientific Catalog #S233-50
**Disposable Filter Flask with PES Membrane** Contributed by users
**gentleMACS C-Tube** Miltenyi Biotec Catalog #130-093-334
**gentleMACS Dissociator** Miltenyi Biotec Catalog #130-093-235
**500um Cell Strainer** pluriSelect Catalog #43-50500-01
**100um Cell Strainer** Fisher Scientific Catalog #22363549
**40um Cell Strainer** Fisher Scientific Catalog #22363547
**15ml Conical Tubes** Olympus Catalog #21103
**Nexcelom Cellometer** Contributed by users
**Nexcelom Cellometer Slides** Contributed by users
**Nexcelom ViaStain AO/PI** Contributed by users
**1mL Cryovials Internally Threaded with O-ring** Olympus Catalog #24-200P
**Cryostar CS10** Sigma Aldrich Catalog #C2874
**Cool Cells** biocision Catalog #BCS405G
**LabXpert Label Maker** Brady Worldwide, Inc. Catalog #060900
**LabXpert Label Maker Stickers** Brady Worldwide, Inc. Catalog #X-120-492

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face mask with shield, gloves, lab coat or apron).

Preparation

1. To make ACK Lysis Buffer:
   1.1 Add 8.02 grams of NH$_4$Cl, 0.84 grams of NaHCO$_3$, and 5uL of 0.5M EDTA into 1000mL of deionized water.
   1.2 Filter buffer through 22um PES Filter. Aliquot in 50mL conicals and store at room temperature for long-term storage.

2. To make the cDMEM:
   2.1 Add 50mL of FBS, 10mL of Penicillin/Streptomycin to 440mL of DMEM.
   2.2 Filter through a filter flask and store at 4°C.

Procedure

3. Obtain 1cm x 1cm x1cm tissue cube (thymus and spleen) or whole/partial lymph node from organ dissection protocol. Place in 15mL conical tube with 5mL cDMEM, retain on ice.

4. Prepare 10mL digest mix using 1mL of 10x Collagenase/Hyaluronidase in DMEM and 9mL dMEM with 5% FBS. Warm to 37°C in water bath.

5. Mince tissue crosswise with two scalpels in sterile petri dish until tissue fragments are approximately 2 mm in all dimensions.

6. Transfer tissue fragments to C tube, incubate at 37°C in shaking incubator for 30 minutes.

7. Place C tube on gentleMACS dissociator, run protocol mspln0201.

8. Transfer cell suspension to 50 ml conical over 500 uM mesh filter and wash over filter with DMEM until tube is full. Centrifuge 350 x g for 10 minutes beginning at RT, step down to 8°C.
9 Discard supernatant. Resuspend cell pellet in cold cDMEM, decant over 100 uM filter into new conical tube. Centrifuge 350 x g for 10 minutes, 8°C.

10 Discard supernatant and retain cell pellet. Resuspend in 10 ml cold HBSS, decant over 40 uM filter into new conical tube. Retain cell suspension on ice while performing cell count.

11 Take a 20ul aliquot for counting on a Nexcelom Cellemeter.

12 Add 20ul of Viastain AO/PI to aliquot and mix thoroughly with pipette. Add 20ul to counting slide and count with “immune cells low RBC” with dilution factor 2.0. Record total yield and viability in case worksheet.

13 Centrifuge remaining cell suspension at 350 x g for 10 minutes at 8°C. Decant supernatant and dissociate cell pellet. Resuspend dropwise in Cryostor 10 at 25 million cells/ml.

14 Aliquot suspension to labeled cryovial and place in CoolCell. Transfer to -80°C freezer for 18-24 hours prior to transfer to liquid nitrogen cryounit.