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We use this protocol and it's working

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HubMap UF TMC Tissue Dissociation to Single Cell

Marda Jorgensen¹, Maigan Brusko¹

¹University of Florida

Human BioMolecular Atlas Program (HuBMAP) Method Development Community

Tech. support email: Jeff.spraggins@vanderbilt.edu

Marda Jorgensen

GUIDELINES

Perform all steps using appropriate aseptic technique.

MATERIALS

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- ✂ Forceps **Gold Biotechnology Catalog #F3001**
- ✂ Scissors **Carl Roth Catalog #HCT7.1**
- ✂ DMEM **Gibco - Thermo Fischer Catalog #11885**
- ✂ Sterile deionized H2O **Contributed by users**
- ✂ 10X Collagenase/Hyaluronidase in DMEM **Stemcell Technologies**
- ✂ 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**

SAFETY WARNINGS

Use universal safety precautions when handling human samples and employ personal protective equipment (e.g., face mask with shield, gloves, lab coat or apron).

Preparation

- 1** To make ACK Lysis Buffer:
 - 1.1** Add 8.02 grams of NH_4Cl , 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 37C 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 Cellometer.
- 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.