Apr 22, 2020

# HubMap UF TMC Tissue Dissociation to Single Cell

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1 Works for me dx.doi.org/10.17504/protocols.io.bd9vi966

Human BioMolecular Atlas Program (HuBMAP) Method Development Community

GUIDELINES

Perform all steps using appropriate aseptic technique.

MATERIALS

| NAME | CATALOG # | VENDOR |
|---|---|---|
| Forceps | F3001 | Gold Biotechnology |
| Scissors | HCT7.1 | Carl Roth |
| DMEM | 11885 | Gibco - Thermo Fischer |
| Sterile deionized H2O | | |
| 10X Collagenase/Hyaluronidase in DMEM | | Stemcell Technologies |
| Miltenyi C Tube | | |
| Sterile Petri Dishes | | |
| Disposable Scalpels 10 Blade | | |
| FBS USDA Origin | 25-550 | |
| Penicillin/Streptomycin | 30-002-CL | Corning |
| 0.5M EDTA | BM-150 | Boston Bioproducts |
| Ammonium Chloride | A661-500 | Fisher Scientific |
| NaHCO | S233-50 | Fisher Scientific |
| Disposable Filter Flask with PES Membrane | | |
| gentleMACS C-Tube | 130-093-334 | Miltenyi Biotec |
| gentleMACS Dissociator | 130-093-235 | Miltenyi Biotec |
| 500um Cell Strainer | 43-50500-01 | pluriSelect |
| 100um Cell Strainer | 22363549 | Fisher Scientific |
| 40um Cell Strainer | 22363547 | Fisher Scientific |
| 15ml Conical Tubes | 21103 | Olympus |
| Nexcelom Cellometer | | |
| Nexecelom Cellometer Slides | | |



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| NAME | CATALOG # | VENDOR |
|---|---|---|
| Nexecelom ViaStain AO/PI | | |
| 1mL Cryovials Internally Threaded with O-ring | 24-200P | Olympus |
| Cryostar CS10 | C2874 | Sigma Aldrich |
| Cool Cells | BCS405G | biocision |
| LabXpert Label Maker | 060900 | Brady Worldwide, Inc. |
| LabXpert Label Maker Stickers | X-120-492 | Brady Worldwide, Inc. |

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, 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.



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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.





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