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Zebrafish Embryo Dissociation for MACS V.2

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We use this protocol and it's working

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Abstract

Method of zebrafish embryo dissociation and microbead binding for magnetic enrichment by MACS.

Guidelines

All steps here may should be optimized for a given transgenic line, and particular stage of development (where cells are more or less sticky, more or less rare, etc.) Steps here were optimized for harvesting cells from 1- 3 dpf embryos expressing either kdrl:hCD4 or runx1+23:hLNGFR.

If assessing efficacy of magnetic enrichment, cross lines into fluorescent reporters for analysis by flow cytometry.

Materials

MATERIALS

- Trypan blue **Bio Basic Inc.** **Catalog #**TT1140.SIZE.10g
- Hemocytometer (Neubauer)
- Razor blade
- Ice
- DMEM **Invitrogen - Thermo Fisher**
- Falcon® 5 mL Round Bottom Polystyrene Test Tube, with Cell Strainer Snap Cap **Corning** **Catalog #**352235
- Thermomixer
- PBS
- FBS
- Tumor Dissociation Kit human **Miltenyi Biotec** **Catalog #**130-095-929
- MACS Buffer (0.5% BSA 2mM EDTA in PBS)
- Microbeads **Miltenyi Biotec**

Other enzyme mixes for dissociation may work as well, however they must preserve the epitopes used for microbead binding. Miltenyi provides a list of preserved epitopes for each of their enzyme kits online. This kit will preserve human CD4 and human LNGFR. Enzymes should be reconstituted and aliquoted at -20C.

Before start

Acquire or derive a transgenic line expressing a human surface peptide for antibody binding, and purchase the appropriate human microbead kit.

1 Prepare enzyme mix by adding 200ul Enzyme H, 100ul Enzyme R, and 25ul Enzyme A to 4.7mL DMEM on ice.

2 Tricaine and transfer embryos in minimal E3 to plate lid. Remove excess E3 (can be done effectively by blocking embryos with razor blade and wicking up water behind the blade with a Kimwipe). Chop embryos in plate lid. Make sure blade is in full contact with bottom surface, rotating the plate lid periodically.

3 Recover in 1.5mL enzyme mix to Eppendorf tube.

4 Vortex and incubate for 15-20 minutes at 37C in thermomixer. Vortex and pipet up and down vigorously every ~5 minutes until sample is dissociated.

5 Add FBS to 10% to quench (~150ul).

6 Filter through 40um blue cap FACS tubes on ice.

7 Transfer to fresh Eppendorf tubes and spin down 4 minutes @ 800rpm (tabletop centrifuge – aprox 60xg).

8 Carefully remove and discard supernatant. Resuspend cells in 1mL cold PBS. Take 1ul and add to 99ul Trypan blue in an Eppendorf to count a 1:100 dilution.

9 Spin down in tabletop centrifuge for 5 minutes at 300xg.

10 While cells are spinning down, mix 1:100 dilution and use 10ul to count total cell number on a hemocytometer.

11 Remove and discard supernatant, resuspend in 60ul MACS Buffer per 10^7 total cells. Add 20ul Microbeads per 10^7 total cells.

12 Mix well and incubate for 15 minutes @ 4C protected from light.

13 Wash by adding 1-2 mL MACS Buffer and centrifuge 5 minutes at 300xg, aspirate supernatant completely.

14 Resuspend up to 10^8 cells in 500ul of MACS Buffer.

15 Proceed to magnetic separation.

These cells are now compatible for use with either the AutoMACS Pro separator or manual column separation.

16 If proceeding to single cell RNA sequencing after magnetic enrichment, cells should be washed with PBS/0.5% BSA, spun down and resuspended before counting on a hemocytometer. EDTA in the MACS buffer can inhibit reverse transcription, so this should be removed.