

Bone marrow immunophenotyping MGP_BMI_001

Purpose

Protocol for isolation of bone marrow and processing into single cell suspension

Equipment

Reagents & Buffers

1. HBSS (Invitrogen14170-138)
2. Fetal Calf Serum (FCS)
3. RBC lysis solution (eBiosciences 00-4300-54, made up to 1× with ddH₂O)
4. FACS buffer (PBS (-Mg/-Ca), 0.5% FCS, 2 mM EDTA)

Materials

1. Forceps & scissors
2. Paper tissues
3. 200 l pipette tips
4. 1.7 ml microfuge tubes
5. 30 µm CellTrics filters (Partec, 04-0042-2316)
6. Dispensing troughs for multichannel pipetting
7. 96-well 350 l polypropylene V-bottom plates (BD Falcon, 353263)

Equipment

1. Table top centrifuge

Procedure

Samples are shipped as whole legs in 50 ml tubes containing HBSS on ice from WTSI to KCL (approximately 2 hours by courier) and processed on the same day.

1. Prepare buffers and antibody master mix (see staining protocol) beforehand. Label plates for staining.
2. Prepare bone marrow extraction tubes – see instructions below protocol.
3. Remove leg from 50 ml tube and remove muscle from the tibia using forceps and scissors. Residual muscle can be removed using paper tissues.
4. Put the leg back into the tube to prevent drying out. Continue until all samples have been prepared up to this step.
 - Note: Once legs have been cleaned; batches are processed (step 5 onwards) in groups of 6 to prevent drying out.

5. One at time, remove leg from tubes again, and cut at the ends of the tibia using bone scissors. The cut points are just below the knee and just above the ankle. Ensure you can see red marrow through the cross section of the cut.
6. Place the rest of the leg back into the 50ml tube for contingency in case insufficient marrow is obtained from the tibia.
7. Insert the bone into the bone marrow extraction tube with the widest end on the bone at the bottom.
8. Six at a time, place microfuge tubes into microfuge and spin at 800×g for 30 seconds.
9. Inspect tubes to ensure bone marrow has been extracted. Otherwise cut the end off the bone to increase the opening and repeat. If no bone marrow can be extracted, repeat protocol with femur after all other samples have been processed.
10. Discard the pipette tips containing the bone from the microfuge.
11. Resuspend the bone marrow pellet in 50 µl of 1× RBC lysis buffer at room temperature. Pipette several times to break up pellet.
12. Incubate for 1 minute at room temperature.
13. Add 200 µl FACS buffer.
 - Note: Doing six at a time, allocating 10 seconds to each tube, 1 minute is just enough to do 6 tubes and to go back to the 1st tube to add FACS buffer.
14. Centrifuge at 500×g for 30 seconds.
15. Remove supernatant and resuspend in 200 l FACS buffer.
16. Repeat steps 4-14, six samples at a time, until all the bones have been processed
17. Filter cells into 1.7ml microfuge tubes using 30 µm filters. Rinse tube and filter using 200 µl FACS buffer.
18. Pipette 200 µl of each sample into a 96-well plate.
19. Centrifuge plate at 800×g for 1 minute at 8° C.
20. Cells are now in single cell suspension on plates and ready for staining (see staining protocol).

Preparation of bone marrow extraction tubes

1. Cut a 200 ml pipette tip at the 10 l line and just below the line where the tip of the pipette would end (see picture, colour for illustration only).
2. Insert the middle section of the cut tip into the top section
3. Place into a 1.7 ml tube.

Parameters and Metadata

Pre-pro B cells (Hardy fraction A) - % of B220+ MGP_BMI_001_001 | v1.1

simpleParameter

Req. Analysis: false

Req. Upload: true

Is Annotated: true

Unit Measured: %

Description: pre_pro_b_cells_hardy_fraction_a_of_b220

Pro-B cells (Hardy fractions B and C) - % of B220+ MGP_BMI_002_001 | v1.2

simpleParameter

Req. Analysis: false

Req. Upload: true

Is Annotated: true

Unit Measured: %

Description: pro_b_cells_hardy_fractions_b_and_c_of_b220

Pro-B cells (Hardy fraction B) - % of B220+ MGP_BMI_003_001 | v1.2

simpleParameter

Req. Analysis: false

Req. Upload: true

Is Annotated: true

Unit Measured: %

Description: pro_b_cells_hardy_fraction_b_of_b220

Pro-B cells (Hardy fraction C) - % of B220+ MGP_BMI_004_001 | v1.1

simpleParameter

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Unit Measured: %

Description: pro_b_cells_hardy_fraction_c_of_b220

Pre-B cells (Hardy fraction D) - % of B220+ MGP_BMI_005_001 | v1.1

simpleParameter

Req. Analysis: false

Req. Upload: true

Is Annotated: true

Unit Measured: %

Description: pre_b_cells_hardy_fraction_d_of_b220

Immature B cells (Hardy fraction E) - % of B220+ MGP_BMI_006_001 | v1.1

simpleParameter

Req. Analysis: false

Req. Upload: true

Is Annotated: true

Unit Measured: %

Description: immature_b_cells_hardy_fraction_e_of_b220

Mature B cells (Hardy fraction F) - % of B220+ MGP_BMI_007_0

01 | v1.1

simpleParameter

Req. Analysis: false

Req. Upload: true

Is Annotated: true

Unit Measured: %

Description: mature_b_cells_hardy_fraction_f_of_b220

Pre-pro B cells (Hardy fraction A) - % of CD45+ MGP_BMI_008_

001 | v1.1

simpleParameter

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Unit Measured: %

Description: pre_pro_b_cells_hardy_fraction_a_of_cd45

Pro-B cells (Hardy fractions B and C) - % of CD45 MGP_BMI_

009_001 | v1.1

simpleParameter

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Unit Measured: %

Description: pro_b_cells_hardy_fractions_b_and_c_of_cd45

Pro-B cells (Hardy fraction B) - % of CD45+ MGP_BMI_010_001 |
v1.1

simpleParameter

Req. Analysis: false **Req. Upload:** true **Is Annotated:** false

Unit Measured: %

Description: pro_b_cells_hardy_fraction_b_of_cd45

Pro-B cells (Hardy fraction C) - % of CD45+ MGP_BMI_011_001 |
v1.1

simpleParameter

Req. Analysis: false **Req. Upload:** true **Is Annotated:** false

Unit Measured: %

Description: pro_b_cells_hardy_fraction_c_of_cd45

Pre-B cells (Hardy fraction D) - % of CD45+ MGP_BMI_012_001 |
v1.1

simpleParameter

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Unit Measured: %

Description: pre_b_cells_hardy_fraction_d_of_cd45

Immature B cells (Hardy fraction E) - % of CD45+ MGP_BMI_0

13_001 | v1.1

simpleParameter

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Unit Measured: %

Description: immature_b_cells_hardy_fraction_e_of_cd45

Mature B cells (Hardy fraction F) - % of CD45+ MGP_BMI_014_0

01 | v1.1

simpleParameter

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Unit Measured: %

Description: mature_b_cells_hardy_fraction_f_of_cd45

Total B cells - % of CD45+ MGP_BMI_015_001 | v1.1

simpleParameter

Req. Analysis: false

Req. Upload: true

Is Annotated: true

Unit Measured: %

Description: total_b_cell_precursors_of_cd45

Granulocytes - % of CD45+ MGP_BMI_016_001 | v1.1

simpleParameter

Req. Analysis: false

Req. Upload: true

Is Annotated: true

Unit Measured: %

Description: granulocyte_precursors_of_cd45

Myeloid cells - % of CD45+ MGP_BMI_017_001 | v1.1

simpleParameter

Req. Analysis: false

Req. Upload: true

Is Annotated: true

Unit Measured: %

Description: myeloid_precursors_of_cd45

Plasma cells - % of CD45+ MGP_BMI_018_001 | v1.1

simpleParameter

Req. Analysis: false

Req. Upload: true

Is Annotated: true

Unit Measured: %

Description: plasma_cells_of_cd45

T cells - % of CD45+ MGP_BMI_019_001 | v1.1

simpleParameter

Req. Analysis: false

Req. Upload: true

Is Annotated: true

Unit Measured: %

Description: t_cells_of_cd45

NK cell - % of CD45+ MGP_BMI_020_001 | v1.1

simpleParameter

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Unit Measured: %

Description: nk_cell_precursors_of_cd45

Pre-pro B cells (Hardy fraction A) - cell count MGP_BMI_021_001
| v1.1

simpleParameter

Req. Analysis: false **Req. Upload:** true **Is Annotated:** false

Description: pre_pro_b_cells_hardy_fraction_a_cell_count

Pro-B cells (Hardy fractions B and C) - cell count MGP_BMI_022_001 | v1.1

simpleParameter

Req. Analysis: false **Req. Upload:** true **Is Annotated:** false

Description: pro_b_cells_hardy_fractions_b_and_c_cell_count

Pro-B cells (Hardy fraction B) - cell count MGP_BMI_023_001 | v1.1

simpleParameter

Req. Analysis: false **Req. Upload:** true **Is Annotated:** false

Description: pro_b_cells_hardy_fraction_b_cell_count

Pro-B cells (Hardy fraction C) - cell count MGP_BMI_024_001 | v1.1

simpleParameter

Req. Analysis: false Req. Upload: true Is Annotated: false

Description: pro_b_cells_hardy_fraction_c_cell_count

Pre-B cells (Hardy fraction D) - cell count MGP_BMI_025_001 | v1.1

simpleParameter

Req. Analysis: false Req. Upload: true Is Annotated: false

Description: pre_b_cells_hardy_fraction_d_cell_count

Immature B cells (Hardy fraction E) - cell count MGP_BMI_026_001 | v1.1

simpleParameter

Req. Analysis: false Req. Upload: true Is Annotated: false

Description: immature_b_cells_hardy_fraction_e_cell_count

Mature B cells (Hardy fraction F) - cell count MGP_BMI_027_001

| v1.1

simpleParameter

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Description: mature_b_cells_hardy_fraction_f_cell_count

Total B cells - cell count MGP_BMI_028_001 | v1.1

simpleParameter

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Description: total_b_cell_precursors_cell_count

Granulocytes - cell count MGP_BMI_029_001 | v1.1

simpleParameter

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Description: granulocyte_precursors_cell_count

Myeloid cells - cell count MGP_BMI_030_001 | v1.1

simpleParameter

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Description: myeloid_precursors_cell_count

Plasma cells - cell count MGP_BMI_031_001 | v1.0

simpleParameter

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Description: plasma_cells_cell_count

T cells - cell count MGP_BMI_032_001 | v1.0

simpleParameter

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Description: t_cells_cell_count

NK cells - cell count MGP_BMI_033_001 | v1.1

simpleParameter

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Description: nk_cell_precursors_cell_count

BM panel singlets cell count MGP_BMI_034_001 | v1.0

simpleParameter

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Description: bm_panel_singlets_cell_count

BM panel live cell count MGP_BMI_035_001 | v1.0

simpleParameter

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Description: bm_panel_live_cell_count

BM panel lymphocyte gate cell count MGP_BMI_036_001 | v1.0

simpleParameter

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Description: bm_panel_lymphocyte_gate_cell_count

BM panel CD45+ cell count MGP_BMI_037_001 | v1.0

simpleParameter

Req. Analysis: false Req. Upload: true Is Annotated: false

Description: bm_panel_cd45_cell_count

3i bone marrow panel MGP_BMI_038_001 | v1.0

procedureMetadata

Req. Analysis: false Req. Upload: true Is Annotated: false

Description: 3i_bone_marrow_panel

Options:

- (A) B220 pe-cy-7, CD43 percp-cy5.5, CD24 PE, bp1/ly51 AF647, igm BV421, igd AF488, gr1 AF700, cd11b BV510, CD3 BV786, CD161 BV650, live/dead near IR,
 - (B) CD45 NC605, B220 pe-cy-7, CD43 percp-cy5.5, CD24 PE, bp1/ly51 AF647, igm BV421, igd AF488, gr1 AF700, cd11b BV510, CD3 BV786, CD161 BV650, live/dead near IR,
 - (C) CD45 qdot605, B220 pe-cy-7, CD43 percp-cy5.5, CD24 APC, bp1/ly51 PE, igm BV421, igd FITC, gr1 AF700, cd11b BV510, CD3 BV786, CD138 BV650, live/dead near IR,
-

FACS equipment name MGP_BMI_039_001 | v1.0

procedureMetadata

Req. Analysis: false Req. Upload: true Is Annotated: false

Description: facs_equipment_name

Options: Flow cytometer,

FACS equipment manufacturer MGP_BMI_040_001 | v1.0

procedureMetadata

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Description: facs_equipment_manufacturer

Options: BD Biosciences, Beckman coulter,

FACS equipment model MGP_BMI_041_001 | v1.0

procedureMetadata

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Description: facs_equipment_model

Options: LSR II, FC500, LSR fortessa X20,

FACS sample status MGP_BMI_042_001 | v1.0

procedureMetadata

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Description: facs_sample_status

Options: Fresh, Fixed, Analysed next day unfixed, Prepared next day, analysed fresh,

Tissue dissociation method MGP_BMI_043_001 | v1.0
procedureMetadata

Req. Analysis: false **Req. Upload:** true **Is Annotated:** false

Description: tissue_dissociation_method

Options: Gentlemacs, Pestle, Centrifugation,

Comment MGP_BMI_044_001 | v1.0
simpleParameter

Req. Analysis: false **Req. Upload:** false **Is Annotated:** false

Description: comment

Images associated with FACS analysis MGP_BMI_045_001 | v1.0
seriesMediaParameter

Req. Analysis: false **Req. Upload:** false **Is Annotated:** false
