Immunophenotyping IMPC_IMM_002

Purpose

This test differentiates immune cell sub-populations via flow cytometry.

Description: increased CD4-positive T cell number (MP:0008074), decreased CD4-positive T cell number (MP:0008075), etc., ...

Experimental Design

- **Minimum number:** 3M + 3F
- **Age at test:** Week 16
- **Sex:** Both (sexually dimorphic)

Equipment

Equipment

- Scissors and forceps for biopsy
- Precision balance
- Calibrated single and multichannel pipettes
- Plate shaker
- Refrigerated centrifuge
- Flow Cytometer (capable of distinguishing a minimum of 8 colours per well)
- Tissue dissociator:
  - GentleMACS tissue dissociator ***OR***
  - Equipment for manual dissociation
- Cell counter equipment:
  - Orflo Moxi-Z Cell counter ***OR***
  - Coulter Vicell XR OR Life Technologies Attune® Flow Cytometer ***OR***
  - Haemocytometer

Supplies

- 96-well V-bottomed plates (Falcon #353263)
- Petri dishes
- Dispensing troughs
- Low retention pipette tips for antibody solutions
- *(if using GentleMACS for dissociation)* C Tubes. It is acceptable to re-use these once.
- 50ml Falcon tubes
- Cell strainers e.g. 70m cell strainers that fit 50ml Falcon tubes (BD Falcon, #352350) ***OR*** 70-80µM Nytex
- Cell counter recipients (i.e., slides/cassettes/etc. for cell counter)
- *(if sample processing delayed)* RPMI 1640

- *(if sample processing on same day)* HBSS, with phenol red
- CS (calf serum)
- PBS with Mg^{2+}, with Ca^{2+} (for enzyme buffer used for DNAse and Collagenase D digestions)
- PBS without Mg^{2+}, without Ca^{2+} (for FACS buffer to be used in all steps subsequent to enzymatic digest)
- EDTA (0.5M stock; final concentration 2mM)
- Digestion enzyme (Collagenase D from Roche #11088858001), stock solution in enzyme buffer (see below), aliquoted and stored at –2 0°C
- DNAse I stock solution (Sigma, #DN25) in enzyme buffer (see below), aliquoted and stored at -20°C
- RBC lysis buffer (eBioscience #00-4300-54 or BD Biosciences #555899, both 10X from manufacturer)
- HEPES (pH 7.2-7.4)

Procedure

This protocol requires several steps in the collection, preparation and analysis of the samples. Each one is detailed separately below.

Reagent preparation

Note that two different PBS solutions are required for the protocol below, one with Ca^{2+} and Mg^{2+}, another without Ca^{2+} and Mg^{2+}.

- **Collection buffer:**
  - (*if spleens are to be processed on the same day*) HBSS with Ca^{2+} and Mg^{2+} and phenol red (e.g. Life Technologies 14170161) ***OR***
  - (*if analysis will be delayed*) RPMI medium with 2% CS added.
- **FACS buffer** (for all steps subsequent to enzymatic digest; stable for up to 1 month in the fridge):
  - PBS 1X *without* Ca^{2+}/Mg^{2+} ***OR***
  - HBSS 1X *without* Ca^{2+}/Mg^{2+}
  - EDTA 2mM
  - 2% (v/v) CS
  - 10mM HEPES, pH 7.2-7.4
- **Brilliant Stain Buffer** (BD 563794; for all steps when two or more brilliant violet antibodies are used to prevent non-specific dye-to-dye interaction)
- **Enzyme buffer** (for DNAse and Collagenase D digestions; Stable for up to 1 month in the fridge):
  - PBS *with* Ca^{2+} and Mg^{2+} ***OR***
  - HBSS 1X *with* Ca^{2+}/Mg^{2+}
  - 2% (v/v) CS;
  - 10mM HEPES, pH 7.2-7.4
- **RBC Lysis buffer**: Prepare a 1X solution in ddH_20 from 10X stock lysis buffer.
- **Stopping buffer** (require 300 µl per sample):
  - 1x PBS without Ca^{2+} and Mg^{2+} or 1X HBSS without Ca^{2+} and Mg^{2+}
  - 0.1 M EDTA (37.5 g/L)

- **Antibody cocktails for Panels 1 & 2**
  - Protect antibodies and prepared cocktails from direct light.
  - Final concentration of antibodies should be determined by titration to ensure saturating amounts of antibody are used. Appropriate amounts of antibodies can be mixed together from the manufacturer's stock solutions and stored for 1 week at 4ºC prior to dilution in FACS buffer immediately before use. Do NOT pre-mix BV antibodies. These should be added fresh to the diluted staining mixure.
  - Each sample will require 50 µl (or up to 100 µl) of diluted 1X antibody cocktail.
  - Antibody cocktails should be gently but thoroughly mixed to ensure homogeneity of the solutions.
  - In order to eliminate aggregated antibodies from your mix, centrifuge each antibody cocktail for 8 min at 20,000x*g* and 8°C prior to staining cells.

- **Antibody Panels**

- Recommended antibody (marker) panels, Panel A for T, NKT and NK cells, Panel B for B, myeloid and NK cells are shown below, along with optional markers that may be used by some centres. Core antibodies are required for upload of data; optional markers are not and are listed in alphabetical order. Clones and fluorochromes used should be uploaded for required and optional markers. Where not indicated, clone and fluorochrome choice is dependent on available detectors and filters on the cytometer used at each centre.

Panel A

Type	Antibody (Marker)	Clone	Fluorochrome
Required	CD5	53-7.3	BV421
Required	CD4	RM4-5	FITC
Required	CD44	IM7	PE
Required	CD8a	53-6.7	PE-CF594
Required	CD25	PC61	PE-Cy7
Required	CD161	PK136	APC
Required	CD62L	MEL-14	APC-Cy7
Required	Live/Dead	-	SytoxBlue

Optional	CD3e	145-2C11	
Optional	CD24	M1/69	
Optional	CD27	LG.3A10	
Optional	CD357/GITR	DTA-1	
Optional	CD45	30-F11	
Optional	KLRG1	2F1	
Optional	Ly6c	AL-21	
Optional	TCRd	GL-3	

Panel B

Type	Antibody (Marker)	Clone	Fluorochrome
Required	CD5	53-7.3	BV421
Required	Ly6G	1A3	BV421
Required	CD19	1D3	BV510
Required	Ly6C	AL-21	FITC
Required	CD21/CD35	7G6	PE
Required	CD11b	M1/70	PE-CF594

Required	CD11c	HL3	PE-Cy7
Required	CD161	PK136	APC
Required	MHCII	M5/114.15.2	APC-Cy7 or A700
Required	Live/Dead	-	SytoxBlue
Optional	CD23	B3B4	
Optional	CD27	LG3.A10	
Optional	CD43	S7	
Optional	CD44		
Optional	CD45	30-F11	
Optional	CD317	927	
Optional	F4/80	BM8	
Optional	IgD		
Optional	KLRG	2F1	

- **Read buffer / dead cell exclusion dye**
    - SytoxBlue at 1:10000 concentration in FACS buffer ***OR***
    - SytoxGreen at 1:20000 concentration in FACS buffer
    - Zombie Near Infra-Red live dead from Biolegend at 1:2000 concentration
    - Require 200 l per well (i.e. 400 l for each spleen).
- **Enzyme cocktail (working solution):** 3 ml for each spleen, containing final concentrations of:
    - DNAse I: 30-100 g (from 10 mg/ml stock in enzyme buffer stored in single experiment aliquots at -20ºC, do not freeze-thaw stock)

- Collagenase D: 600 Mandl Units (from 30 U/µl stock in enzyme buffer stored in single experiment aliquots at -20ºC, do not freeze-thaw stock)

NOTE**:** To top up to the 3ml use enzyme buffer; any intermediate dilutions of the enzyme stock solutions should be prepared with enzyme buffer.

Other preparations on the day

- Bring RBC lysis buffer and stop solution to room temperature.
- Prepare wet ice box, label tubes, etc.

Note all centrifuge steps are: 5 min, 400 x g at 8°C

Spleen collection

- Collect the spleen from euthanized mice.
- Remove all fat from the spleen and weigh the organ on a petri dish (do not hydrate the organ before weighing it as this would lead to substantial errors in measurement).
- Place the spleen in a 1.5ml eppendorf tube with 1 mL of sample collection buffer on ice. Use:
  - *(if spleens are to be processed on the same day)* HBSS without calcium, without magnesium but with phenol red ***OR***
  - *(if analysis will be delayed)* RPMI with 2% CS buffer.

Spleen dissociation / digests

If using a GentleMacs tissue dissociator:

- Add the spleen to a GentleMACS C tube containing 3 ml of 1X enzyme cocktail.
- Clip the tube on GentleMACS dissociator and run programme spleen_2.
- Incubate cell suspension for 30 minutes with gentle mixing at least every 5 minutes. Register incubation temperature.
- Run programme spleen_3.
- Add 300 L of stopping buffer and mix by inversion to block enzymatic digestion and dissociate T cell-dendritic cell interactions.
- Filter cell suspension:
  - through 70-80 m Nylon mesh filter into a 50 mL Falcon tube ***OR***
  - directly from C-tubes pour splenocyte suspension through 30 µm CellTrics Partec filters (#04-0042-2316) into 15 ml tubes.
- *(optional)* Wash the GentleMACS C tube with 5ml FACS buffer, filter and pool with flow-through from previous step.
- Centrifuge for 5 minutes, 400 x g at 8°C and discard supernatant.
- Resuspend total splenocytes in 1 mL cold FACS buffer and keep on ice (this step is not required if counting is performed on the attune).

OR, if performing manual digests:

- Place weighed spleen in 12x75mm tube containing 1ml of collagenase solution in 1X HBSS with Ca^{2+} and Mg^{2+} (17-0.2 Wünsch unit/ml)
- Mince into fine pieces using small scissors, place on ice until all samples are minced.

- Add 2ml collagenase (17-0.2 Wünsch unit/ml) to each tube and place in a 37ºC water bath for 30 minutes.
- Tricturate (pipetting vigorously up and down using a 1 mL pipetman) the mixture to break up clumps.
- Spin at 500 x g in a swing bucket rotor for 5 min at 10ºC. Decant the supernatant, rack the tubes or vortex to resuspend the pellet. Add 2ml FACS buffer, mix well by vortexing, take 10 µl for the counting step.
- Dilutions for counting: 2 serial 1:10 dilutions (10µl cells + 90µl FACS buffer, then 10µl of the 1:10 dilution + 90µl buffer.)
- Spin for 5min, 500 x g at 10ºC, decant supernatant, blot the top of the tube, resuspend pellet at $1x10^8$ cells/ml.

Cell counting

- Perform a cell count on an aliquot of the re-suspended cells (adjust concentration according to the cell counter method used).
- Note the cell count, correct for dilution and calculate the concentration in cells per µl.
- Cell count:
  - *If performed before RBC lysis*, pipette the volume containing approximately 4 million cells/well to a 96 well plate in horizontal fashion starting from A1 onwards for panel 1 staining.
  - *If performed after RBC lysis*, pipette the volume containing approximately 1-2 million cells/well to a 96 well plate in horizontal fashion starting from A1 onwards for panel 1 staining.
- Do the same for panel 2 staining in separate wells leaving a few empty rows between the panels to avoid cross contamination.
- Top up to final volume of 100 µl using FACS buffer, centrifuge, discard supernatant and keep plate on wet ice.

Red blood cell lysis, blocking & staining

- Remove plate from ice and add 30 to 100 µl of 1X RBC lysis buffer (at room temperature) to each cell pellet from the previous step.
- Pipette up and down 2-3 times to break up the pellet and ensure complete lysis. Alternatively, vortex the edges of the plates, then pipet quickly once to ensure resuspension is ideal for optimal lysis.
- Incubate for 1 minute at room temperature and then return to ice and add 100 to 200 µl of FACS buffer (to stop lysis) to each well.

Note: *Following RBC lysis, every centrifugation step can be performed at 2000rpm for 1 minute in a 96 well plate, which significantly speeds up the protocol. Do take care to resuspend the cells very well to prevent HTS clumping.*

- Centrifuge, discard supernatant and resuspend in 200 µl FACS buffer (this step is not required if lysis was performed in 30 µl, since there will be enough volume left in the well for a bigger wash of 200 µl; saves time on a spin).
- Again centrifuge and discard supernatant and resuspend in 50 µl of 1:100 Fc block and incubate on ice for 10 min. Top up to 200 µl using FACS buffer after incubation.

- Take antibody (AB) cocktails from the fridge. In order to eliminate aggregated ABs from your mix before use, centrifuge each AB cocktail for 8 min at 20,000 x g and 4°C. Dilute antibody cocktail to final working concentration with FACS buffer, or Brilliant stain buffer when two or more brilliant violet antibodies are used, to make the AB mix.
- Centrifuge plate, discard supernatant and resuspend in 50 to 100 µl 1X AB mix in appropriate wells for individual panels followed by incubation on ice and in the dark for 20 min.
- If using Sytox Blue/Sytox Green as live/dead discriminator:
    - Top up to 200 µl with FACS or Brilliant Stain buffer after incubation. Centrifuge, discard supernatant and resuspend in 200 µl FACS or Brilliant Stain buffer.
    - When ready to read plate, centrifuge again and discard supernatant. Resuspend the pellet in 200 µl of read buffer (Sytox Blue diluted 1:10000 in FACS buffer; Sytox Green diluted 1:20000 in FACS buffer).

- If using Zombie NIR dye as live/dead discriminator:
    - Add 200 µl of PBS (RT) to all samples
    - Spin at 2000 rpm for 1 minute 8°C
    - Add 100 µl/well of Zombie Near-IR Live/Dead dye (1/2000) made up in PBS incubate at room temperature for 10 mins, add 200 µl FACS buffer.

General Recommendations for Setting up Cytometer

Set up the analyser to aim acquire 300,000 viable events (live cells) for each of Panels 1 and 2. 500,000 are recommended for panel 2 in order to increase robustness of myeloid population assessment for low frequency populations (macrophages, DCs).

Notes

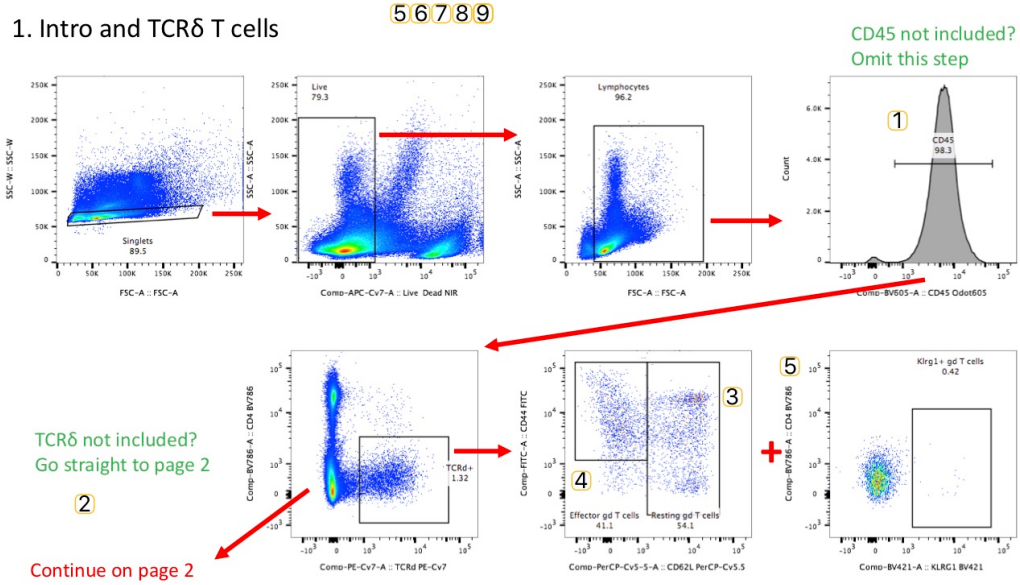
Visual help for Gating

APPENDIX 1. GATING HIERARCHIES

Panel A. Page 1

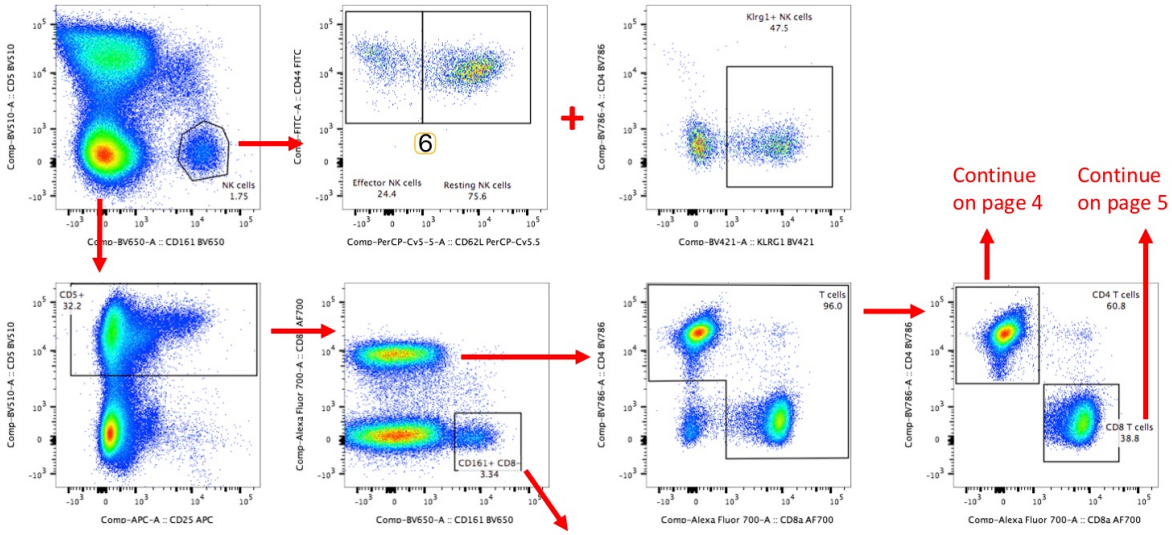
1. Intro and TCRδ T cells

5 6 7 8 9



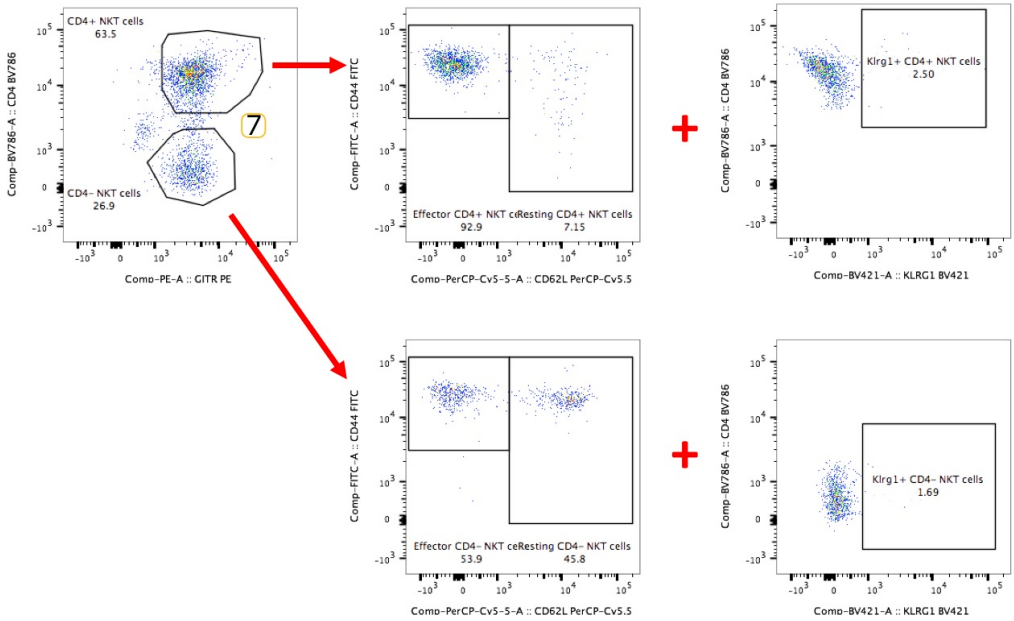
Panel A. Page 2.

2. NK cells and further gating



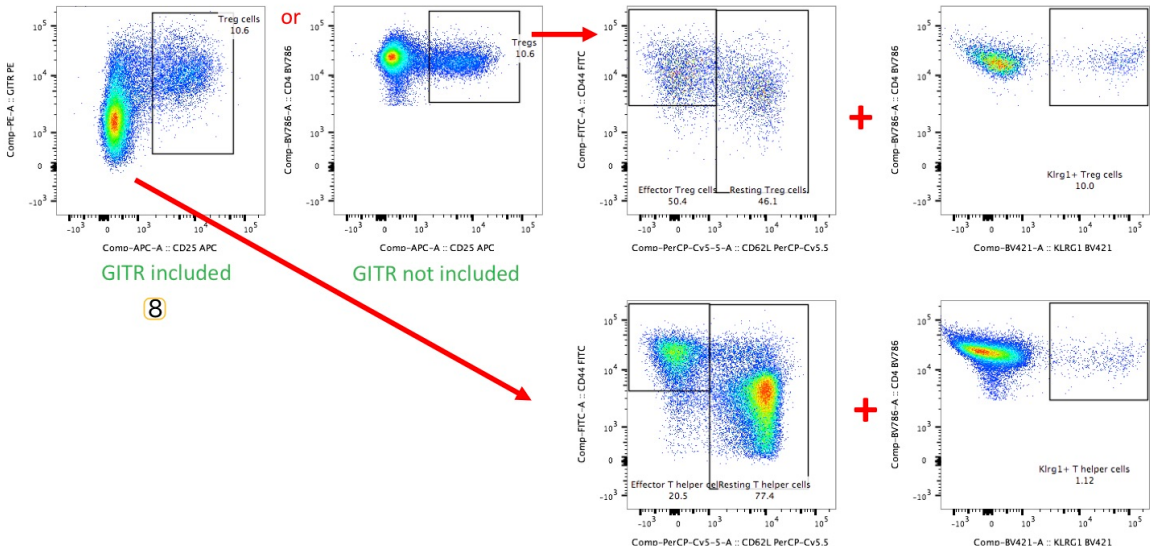
Panel A. Page 3.

3. NKT cells



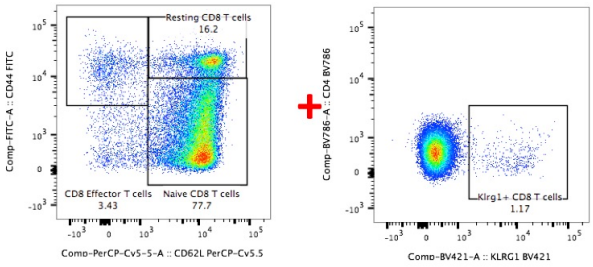
Panel A. Page 4.

4. Tregs and T helper cells



Panel A. Page 5.

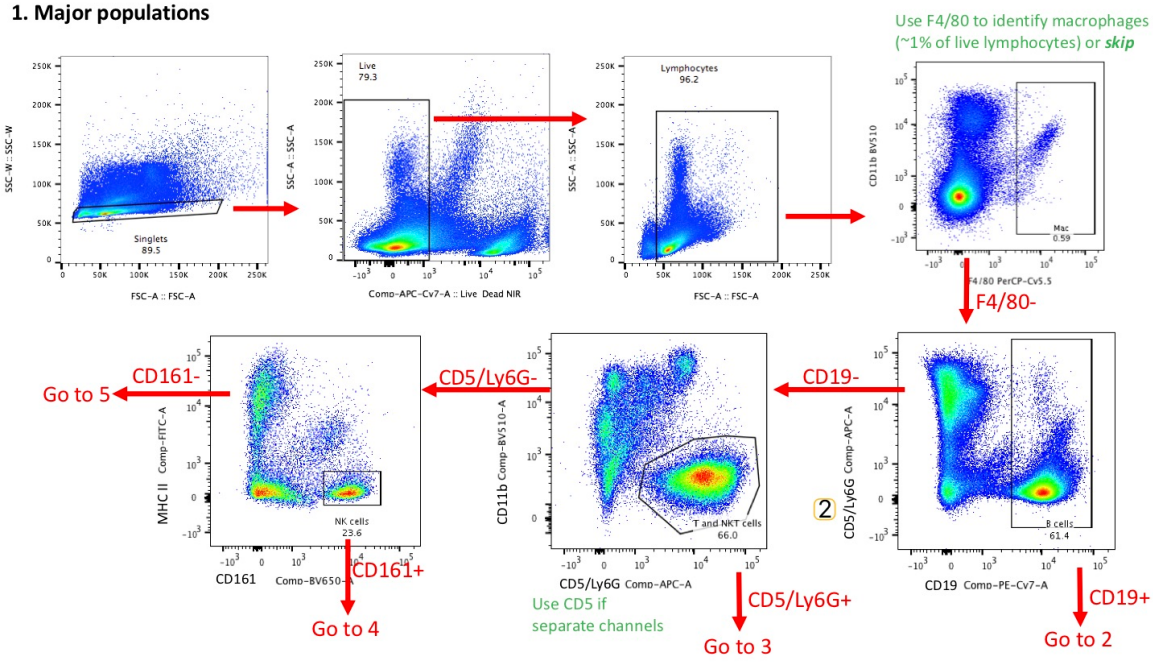
5. CD8 T cells and notes



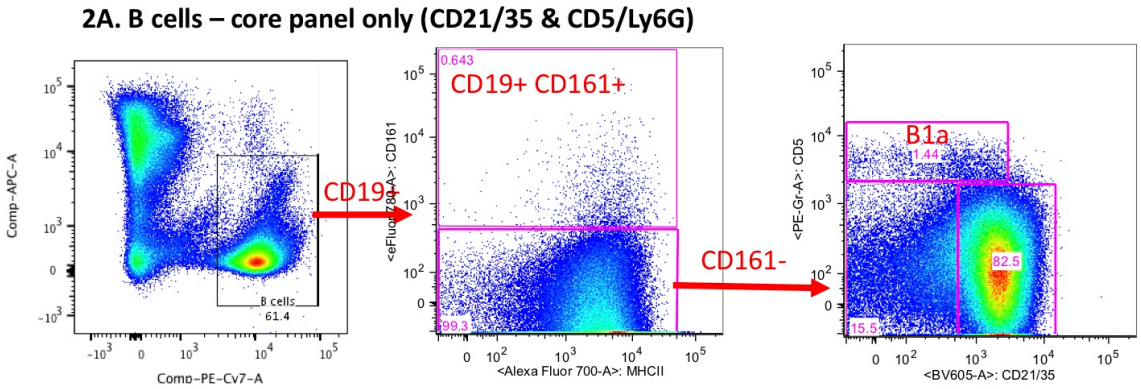
1. If there is no CD45 in the panel, omit this step.
2. Approximately 50% of γδ T cells are CD5-, so they will drop out when gating on CD5 later on. Of the remaining cells, approximately 90% are CD4- CD8- and will drop out of the T cell gate. Only 5% (approximately 0.2% of lymphocytes) will end up in the CD8 T cell gate which is negligible .
3. Please note that each cell type requires different thresholds for both CD44 and CD62L.
4. CD44- CD62L- cells do not occur naturally and show up when CD62L is shed from resting cells during sample preparation.
5. I have chosen CD4 for the y axis because gives a nice compact population for almost all cell types which makes it easy to see the Klrg1+ cells. However, if CD4 doesn't work for you because of your fluorochrome combinations, it can be substituted by any other marker.
6. The name effector is fine for CD4 and CD8 T cells, it is a bit unusual for γδ T cells, NKT cells and NK cells. We settled for this term in the end and also added these population names (with a more detailed description) to the MGI ontology, so MP terms that we use now carry these names.
7. These need to be added up to give the counts of total NKT cells. Use any fluorochrome on the y axis that gates out the non-specific autofluorescent population between the two distinct populations
8. If you don't have GITR, use CD4 on the y axis instead. It works almost as well.

Panel B. Page 1.

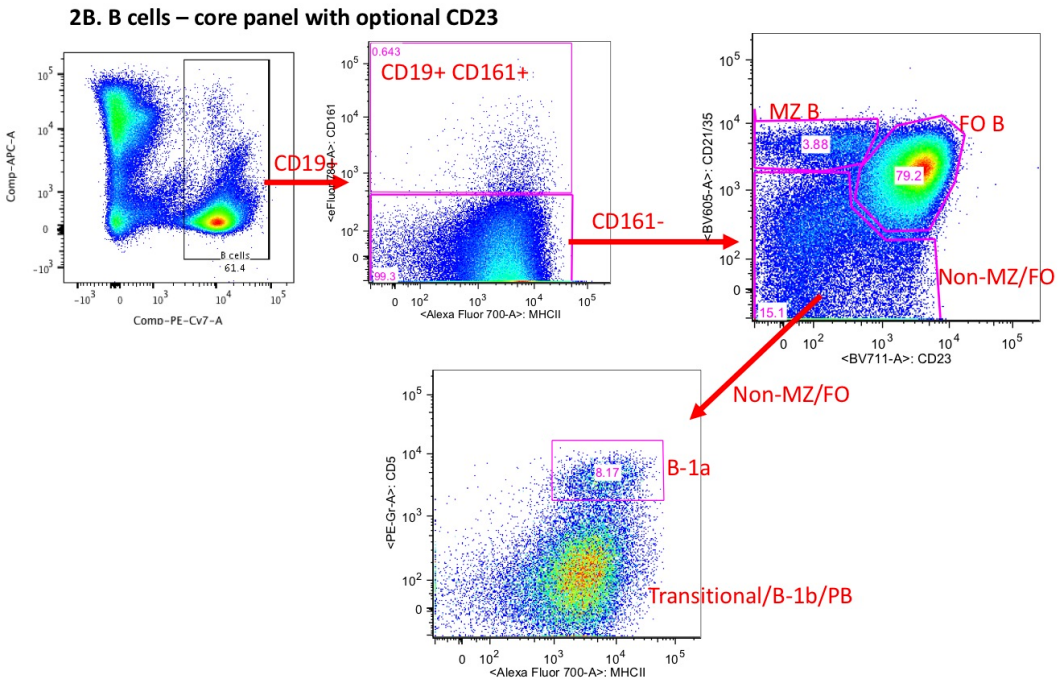
1. Major populations



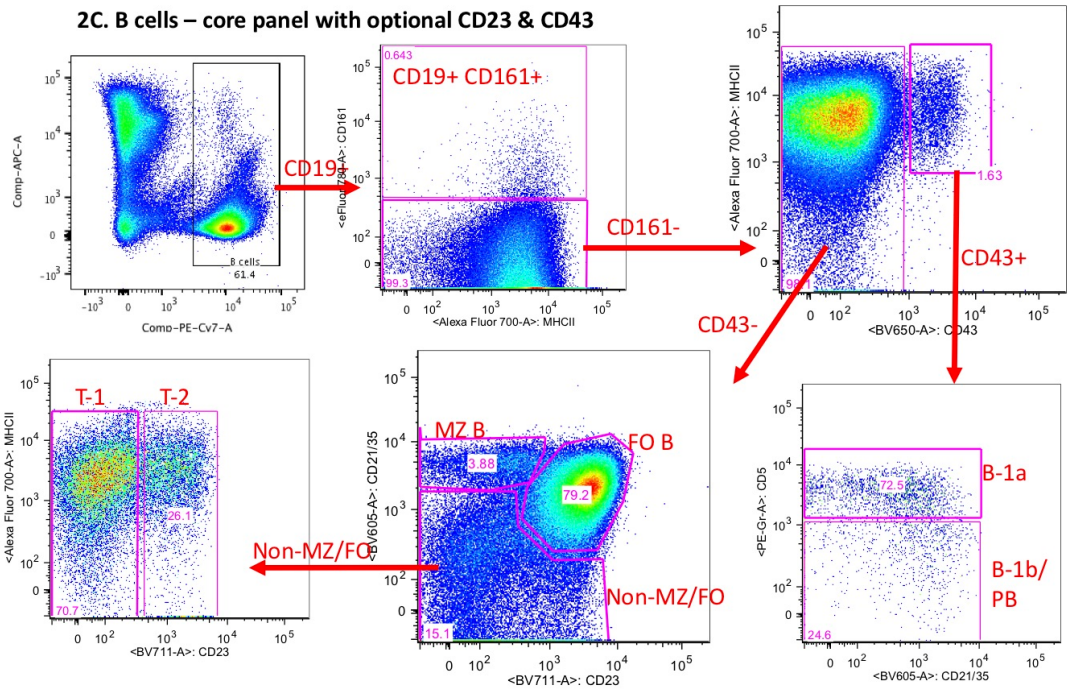
Panel B. Page 2A.



Panel B. Page 2B.

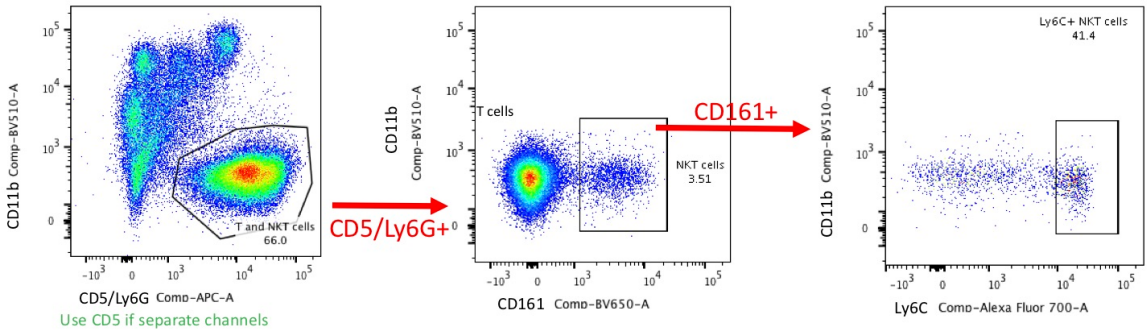


Panel B. Page 2C.



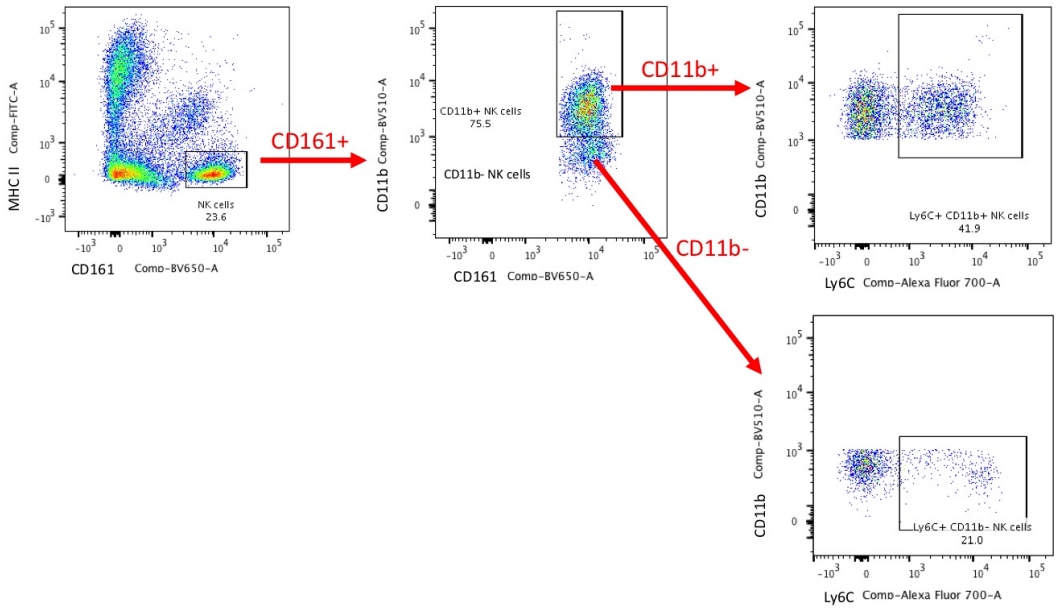
Panel B. Page 3.

3. T cells and NK T cells

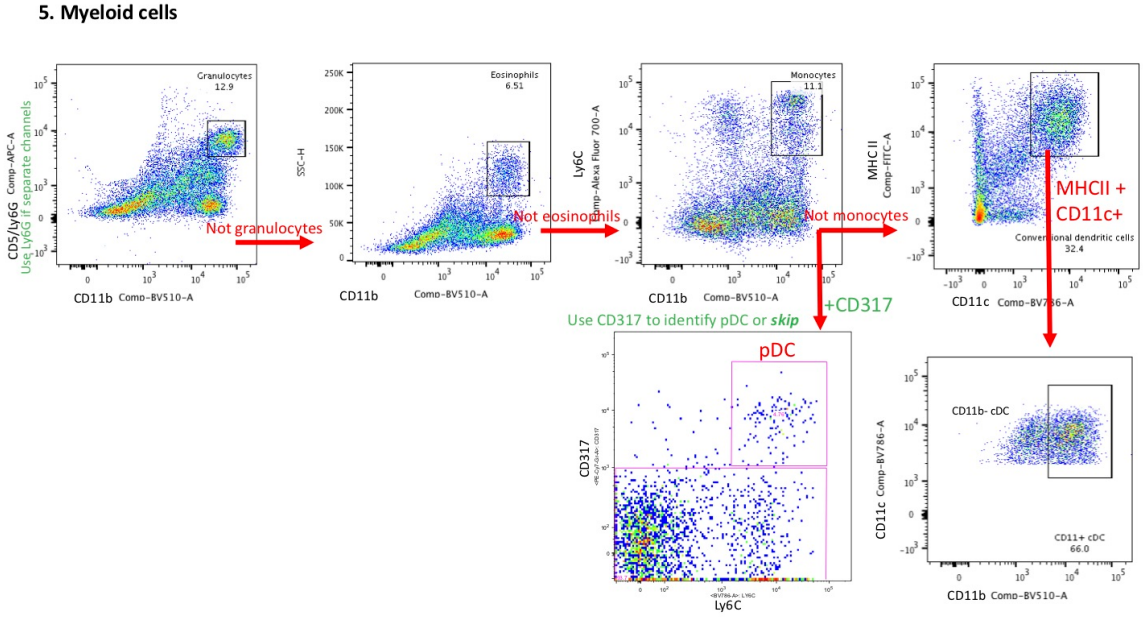


Panel B. Page 4.

4. NK cells



Panel B. Page 5.



Parameters and Metadata

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Live leukocytes (Panel A) - % of total events IMPC_IMM_002_002 | v2.0

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T cells (Panel A) - % of live leukocytes (Panel A) IMPC_IMM_003_002 | v2.0

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NKT cells (panel A) - % of live leukocytes (Panel A) IMPC_IMM_004_002 | v2.0

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NK cells (Panel A) - % of live leukocytes (Panel A) IMPC_IMM_005_002 | v2.0

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CD4 T cells - % of live leukocytes (Panel A) IMPC_IMM_007_002 | v2.0

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CD4+ T helper cells - % of live leukocytes (Panel A) IMPC_IMM_015_002 | v2.0

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Total events (Panel A) IMPC_IMM_026_002 | v2.0

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Total events (Panel B) IMPC_IMM_027_002 | v2.0

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Effector CD4+ T helper cells - % of live leukocytes (Panel A) IMPC_IMM_028_002 | v2.0

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Resting CD4+ T helper cells - % of live leukocytes (Panel A) IMPC_IMM_029_002 | v2.0

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Resting CD8+ T cells - % of live leukocytes (Panel A) IMPC_IMM_034_002 | v2.0

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Effector CD4+ NKT cells - % of live leukocytes (Panel A) IMPC_IMM_040_002 | v2.0

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Unit Measured: %

Resting CD4+ NKT cells - % of live leukocytes (Panel A) IMPC_IMM_041_002 | v2.0

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Effector CD4- NKT cells - % of live leukocytes (Panel A) IMPC_IMM_046_002 | v2.0

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Resting CD4- NKT cells - % of live leukocytes (Panel A) IMPC_IMM_047_002 | v2.0

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Live leukocytes (Panel B) - % of total events (Panel B) IMPC_IMM_049_002 | v2.0

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Granulocytes - % of live leukocytes (Panel B) IMPC_IMM_050_002 | v2.0

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Monocytes - % of live leukocytes (Panel B) IMPC_IMM_051_002 | v2.0

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Eosinophils - % of live leukocytes (Panel B) IMPC_IMM_052_002 | v2.0

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NK cells (Panel B) - % of live leukocytes (Panel B) IMPC_IMM_053_002 | v2.0

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CD11b- NK cells - % of live leukocytes (Panel B) IMPC_IMM_056_002 | v2.0

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CD11b+ NK cells - % of live leukocytes (Panel B) IMPC_IMM_057_002 | v2.0

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NKT cells (panel B) - % of live leukocytes (Panel B) IMPC_IMM_058_002 | v2.0

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Ly6C+ NKT cells - % of live leukocytes (Panel B) IMPC_IMM_059_002 | v2.0

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T cells (panel B) - % of live leukocytes (Panel B) IMPC_IMM_061_002 | v2.0

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Unit Measured: %

B cells - % of live leukocytes (Panel B) IMPC_IMM_063_002 | v2.0

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Unit Measured: %

Follicular B cells - % of B cells (Panel B) IMPC_IMM_067_002 | v2.0

simpleParameter

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

Unit Measured: %

Marginal zone B cells - % of B cells (Panel B) IMPC_IMM_071_002 | v2.0

simpleParameter

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

Unit Measured: %

Conventional DC - % of live leukocytes (Panel B) IMPC_IMM_072_002 | v2.0

simpleParameter

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

Unit Measured: %

Plasmacytoid DC- % of live leukocytes (Panel B) IMPC_IMM_074_002 | v2.0

simpleParameter

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

Unit Measured: %

Macrophages- % of live leukocytes (Panel B) IMPC_IMM_075_002 | v2.0

simpleParameter

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

Unit Measured: %

Equipment name IMPC_IMM_077_002 | v2.0

procedureMetadata

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

Options: FACS, Flow cytometer, Fortessa_1, LSR II, Fortessa_I Custom Build,

---

Equipment manufacturer IMPC_IMM_078_002 | v2.0

procedureMetadata

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

Options: BD Biosciences, Beckman Coulter, IntelliCyt, Cytek,

Equipment model IMPC_IMM_079_002 | v2.0

procedureMetadata

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

Options: BD LSR-II, BD LSRFortessa Cell Analyzer, CANTO-II, FACSAria III, Gallios, H47100123, iQue Screener PLUS, Aurora,

---

CS&T Bead lot IMPC_IMM_080_002 | v2.0

procedureMetadata

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

---

Anesthesia IMPC_IMM_081_002 | v2.0

procedureMetadata

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

Options: Injection narcosis with Ketamine (100mg/kg)/Xylazine (10mg/kg), Injection narcosis with Sodium Pentobarbital (Somnopentyl), Injection narcosis with Tribromoethanol (Avertin), Isoflurane, none, Injection narcosis with Medetomidine/Midazolam/Butorphanol,

Cell digestion IMPC_IMM_082_002 | v2.0

procedureMetadata

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

Options: GentleMACS, manual,

Cell digestion agent IMPC_IMM_083_002 | v2.0

procedureMetadata

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

Options: Collagenase D, Collagenase II, Spleen dissociation kit, manual,

Cell digestion agent manufacturer IMPC_IMM_084_002 | v2.0

procedureMetadata

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

Options: Gibco, Roche, Worthington, Miltenyi Biotec, manual, Sigma,

Cell digestion agent catalog number IMPC_IMM_085_002 | v2.0

procedureMetadata

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

Options: #11088858001, 17101-015, CLS2LS004176, 130-095-926, manual, C6885,

---

Cell counting performed IMPC_IMM_086_002 | v2.0

procedureMetadata

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

Options: post-lysis, pre-lysis,

Cell counting equipment manufacturer IMPC_IMM_087_002 | v2.0

procedureMetadata

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

Options: American Optical, BD Biosciences, Beckman Coulter, Life Technologies, Merck Millipore, Orflo, IntelliCyt, Nextcelom,

---

Cell counting equipment model IMPC_IMM_088_002 | v2.0

procedureMetadata

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

Options: 4468770, Attune, BD LSR-II, Countess Automated Cell Counter, Gallios, Moxi Z, Reichert Brightline, Scepter, Cellometer Auto T4, iQue Screener PLUS,

---

Cell counting equipment name IMPC_IMM_089_002 | v2.0

procedureMetadata

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

Options: Cellometer Auto T4,

Cell lysis buffer manufacturer IMPC_IMM_090_002 | v2.0

procedureMetadata

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

Options: BD PharmLyse, eBioscience, Jax, JMC, LONZA, In house,

---

Cell lysis buffer catalog number IMPC_IMM_091_002 | v2.0

procedureMetadata

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

Options: 00-4300-54, 10-548E, 555899, home brew, In house,

Date and time of sacrifice IMPC_IMM_092_002 | v2.0

procedureMetadata

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

Date and time of sample preparation IMPC_IMM_093_002 | v2.0

procedureMetadata

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

Sample storage temperature until analysis (in Celsius) IMPC_IMM_094_002 | v2.0

procedureMetadata

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

Unit Measured: C

Options: 8,

FCS repository reference (URL/ID) IMPC_IMM_095_002 | v2.0

procedureMetadata

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

---

Balanced salt solution type IMPC_IMM_096_002 | v2.0

procedureMetadata

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

Options: HBSS, PBS, KDS BSS,

Balanced salt solution manufacturer IMPC_IMM_097_002 | v2.0

procedureMetadata

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

Options: Biochrom, Gibco, Life Technologies, Sigma, Wako, Wisent, home brew, In house,

---

Balanced salt solution catalog number IMPC_IMM_098_002 | v2.0

procedureMetadata

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

Options: 041-20211, 14175-095, 14190-144, D1408, H6136-1L, HBSS 1X 14170-088, L 182-10, home brew, 14190169, In house,

---

RPMI manufacturer IMPC_IMM_099_002 | v2.0

procedureMetadata

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

Options: Gibco, Jax, Life Technologies, none used, Sigma, Wako, Thermo Fisher Scientific,

---

RPMI catalog number IMPC_IMM_100_002 | v2.0

procedureMetadata

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

Options: 11875-093, 11875-101, 189-02145, 31800-022, home brew, none used, R8758,

---

DNAse I manufacturer IMPC_IMM_101_002 | v2.0

procedureMetadata

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

Options: Sigma, Spleen Dissociation Kit, N/A,

DNAse I catalog number IMPC_IMM_102_002 | v2.0

procedureMetadata

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

Options: D8764, DN25, N/A, none used,

Dead cell exclusion dye IMPC_IMM_103_002 | v2.0

procedureMetadata

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

Options: DAPI, Propidium Iodide, Sytox Blue, Sytox Green, Zombie NIR, LIVE/DEAD Fixable Aqua stain, Ghost Dye UV450,

---

Dead cell exclusion dye manufacturer IMPC_IMM_104_002 | v2.0

procedureMetadata

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

Options: Biolegend, home brew, Life Technologies, Sigma, Invitrogen by Thermo Fisher, Tonbo biosciences,

---

Dead cell exclusion dye catalog number IMPC_IMM_105_002 | v2.0

procedureMetadata

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

Options: 423106, D9542, home brew, R37606, S-34860, S11348, S34857, P4170, L34966, 13-0868-T500,

---

Cell digestion temperature (in Celsius) IMPC_IMM_106_002 | v2.0

procedureMetadata

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

Options: 37, RT, N/A,

Panel A FCS file(s) IMPC_IMM_107_002 | v2.0

seriesMediaParameter

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

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Panel B FCS file(s) IMPC_IMM_108_002 | v2.0

seriesMediaParameter

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

---

Automated analysis IMPC_IMM_109_002 | v2.0

procedureMetadata

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

Options: No, Yes,

Collection buffer manufacturer IMPC_IMM_110_002 | v2.0

procedureMetadata

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

Options: Life Technologies, BD Biosciences, Wako, home brew,

Collection buffer catalog number number IMPC_IMM_111_002 | v2.0

procedureMetadata

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

Options: 24020, 563503, 084-08965,

FACS buffer manufacturer IMPC_IMM_112_002 | v2.0

procedureMetadata

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

Options: Life Technologies, In house, Wako, home brew,

FACS buffer catalog number IMPC_IMM_113_002 | v2.0

procedureMetadata

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

Options: 14175, In house, 048-29805, home brew,

Enzyme buffer manufacturer IMPC_IMM_114_002 | v2.0

procedureMetadata

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

Options: Life Technologies, N/A, Wako, Miltenyi Biotec,

Enzyme buffer catalog number IMPC_IMM_115_002 | v2.0

procedureMetadata

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

Options: 14025, N/A, 084-08965, 130-095-926,

Total spleen leukocyte count IMPC_IMM_116_001 | v1.0

simpleParameter

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

---

Clog- events (Panel A) IMPC_IMM_117_001 | v1.0

simpleParameter

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

---

FSC/SSC Singlets (Panel A) IMPC_IMM_118_001 | v1.0

simpleParameter

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

---

Effector NK cells - % of live leukocytes (Panel A) IMPC_IMM_119_001 | v1.0

simpleParameter

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

Unit Measured: %

Effector Treg cells - % of live leukocytes (Panel A) IMPC_IMM_120_001 | v1.0

simpleParameter

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

Unit Measured: %

Effector T cells - % of live leukocytes (Panel A) IMPC_IMM_121_001 | v1.0

simpleParameter

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

Unit Measured: %

Klrg1+ CD4- NKT cells - % of live leukocytes (Panel A) IMPC_IMM_122_001 | v1.0

simpleParameter

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

Unit Measured: %

Klrg1+ CD4+ NKT cells - % of live leukocytes (Panel A) IMPC_IMM_123_001 | v1.0

simpleParameter

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

Unit Measured: %

Klrg1+ CD4+ T helper cells - % of live leukocytes (Panel A) IMPC_IMM_124_001 | v1.0

simpleParameter

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

Unit Measured: %

Klrg1+ CD8 T cells - % of live leukocytes (Panel A) IMPC_IMM_125_001 | v1.0

simpleParameter

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

Unit Measured: %

Klrg1+ NK cells - % of live leukocytes (Panel A) IMPC_IMM_126_001 | v1.0

simpleParameter

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

Unit Measured: %

Klrg1+ Treg cells - % of live leukocytes (Panel A) IMPC_IMM_127_001 | v1.0

simpleParameter

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

Unit Measured: %

Klrg1+ T cells - % of live leukocytes (Panel A) IMPC_IMM_128_001 | v1.0

simpleParameter

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

Unit Measured: %

Resting NK cells - % of live leukocytes (Panel A) IMPC_IMM_129_001 | v1.0

simpleParameter

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

Unit Measured: %

Resting Treg cells - % of live leukocytes (Panel A) IMPC_IMM_130_001 | v1.0

simpleParameter

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

Unit Measured: %

Resting T cells - % of live leukocytes (Panel A) IMPC_IMM_131_001 | v1.0

simpleParameter

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

Unit Measured: %

T cells - % of live leukocytes (Panel A) IMPC_IMM_132_001 | v1.0

simpleParameter

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

Unit Measured: %

T cells - % of live leukocytes (Panel A) IMPC_IMM_133_001 | v1.0

simpleParameter

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

Unit Measured: %

CD4- NKT cells - % of NKT cells (Panel A) IMPC_IMM_134_001 | v1.0

simpleParameter

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

Unit Measured: %

CD4+ NKT cells - % of NKT cells (Panel A) IMPC_IMM_135_001 | v1.0

simpleParameter

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

Unit Measured: %

CD4+ T cells - % of T cells IMPC_IMM_136_001 | v1.0

simpleParameter

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

Unit Measured: %

CD4+ T helper cells - % of CD4 T cells IMPC_IMM_137_001 | v1.0

simpleParameter

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

Unit Measured: %

CD8+ T cells - % of T cells IMPC_IMM_138_001 | v1.0

simpleParameter

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

Unit Measured: %

Effector CD4- NKT cells - % of CD4- NKT cells IMPC_IMM_139_001 | v1.0

simpleParameter

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

Unit Measured: %

Effector CD4+ NKT cells - % of CD4+ NKT cells IMPC_IMM_140_001 | v1.0

simpleParameter

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

Unit Measured: %

Effector CD4+ T helper cells - % of CD4+ T helper cells IMPC_IMM_141_001 | v1.0

simpleParameter

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

Unit Measured: %

Effector CD8+ T cells - % of CD8+ T cells IMPC_IMM_142_001 | v1.0

simpleParameter

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

Unit Measured: %

Effector NK cells - % of NK cells (Panel A) IMPC_IMM_143_001 | v1.0

simpleParameter

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

Unit Measured: %

Effector Treg cells - % of Treg cells IMPC_IMM_144_001 | v1.0

simpleParameter

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

Unit Measured: %

Effector T cells - % of T cells IMPC_IMM_145_001 | v1.0

simpleParameter

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

Unit Measured: %

Klrg1+ CD4- NKT cells - % of CD4- NKT cells IMPC_IMM_146_001 | v1.0

simpleParameter

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

Unit Measured: %

Klrg1+ CD4+ NKT cells - % of CD4+ NKT cells IMPC_IMM_147_001 | v1.0

simpleParameter

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

Unit Measured: %

Klrg1+ CD4+ T helper cells - % of CD4+ T helper cells IMPC_IMM_148_001 | v1.0

simpleParameter

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

Unit Measured: %

Klrg1+ CD8 T cells - % of CD8+ T cells IMPC_IMM_149_001 | v1.0

simpleParameter

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

Unit Measured: %

Klrg1+ NK cells - % of NK cells (Panel A) IMPC_IMM_150_001 | v1.0

simpleParameter

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

Unit Measured: %

Klrg1+ Treg cells - % of Treg cells IMPC_IMM_151_001 | v1.0

simpleParameter

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

Unit Measured: %

Klrg1+ T cells - % of T cells IMPC_IMM_152_001 | v1.0

simpleParameter

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

Unit Measured: %

Naïve CD8+ T cells - % of CD8+ T cells IMPC_IMM_153_001 | v1.0

simpleParameter

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

Unit Measured: %

Resting CD4- NKT cells - % of CD4- NKT cells IMPC_IMM_154_001 | v1.0

simpleParameter

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

Unit Measured: %

Resting CD4+ NKT cells - % of CD4+ NKT cells IMPC_IMM_155_001 | v1.0

simpleParameter

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

Unit Measured: %

Resting CD4+ T helper cells - % of CD4+ T helper cells IMPC_IMM_156_001 | v1.0

simpleParameter

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

Unit Measured: %

Resting CD8+ T cells - % of CD8+ T cells IMPC_IMM_157_001 | v1.0

simpleParameter

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

Unit Measured: %

Resting NK cells - % of NK cells (Panel A) IMPC_IMM_158_001 | v1.0

simpleParameter

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

Unit Measured: %

Resting Treg cells - % of Treg cells IMPC_IMM_159_001 | v1.0

simpleParameter

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

Unit Measured: %

Resting T cells - % of T cells IMPC_IMM_160_001 | v1.0

simpleParameter

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

Unit Measured: %

Treg cells - % of CD4 T cells IMPC_IMM_161_001 | v1.0

simpleParameter

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

Unit Measured: %

Clog- events (Panel B) IMPC_IMM_162_001 | v1.0

simpleParameter

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

---

FSC/SSC Singlets (Panel B) IMPC_IMM_163_001 | v1.0

simpleParameter

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

---

B1a cells - % of B cells (Panel B) IMPC_IMM_164_001 | v1.0

simpleParameter

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

Unit Measured: %

B1b cells - % of B cells (Panel B) IMPC_IMM_165_001 | v1.0

simpleParameter

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

Unit Measured: %

CD11b-high cDC - % of conventional DC (Panel B) IMPC_IMM_166_001 | v1.0

simpleParameter

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

Unit Measured: %

CD11b-low cDC - % of conventional DC (Panel B) IMPC_IMM_167_001 | v1.0

simpleParameter

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

Unit Measured: %

CD161+ B cells - % of live leukocytes (Panel B) IMPC_IMM_168_001 | v1.0

simpleParameter

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

Unit Measured: %

Transitional 1 B cells - % of B cells (Panel B) IMPC_IMM_169_001 | v1.0

simpleParameter

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

Unit Measured: %

Transitional 2 B cells - % of B cells (Panel B) IMPC_IMM_170_001 | v1.0

simpleParameter

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

Unit Measured: %

CD11b- NK cells - % of NK cells (Panel B) IMPC_IMM_171_001 | v1.0

simpleParameter

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

Unit Measured: %

CD11b+ NK cells - % of NK cells (Panel B) IMPC_IMM_172_001 | v1.0

simpleParameter

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

Unit Measured: %

CD161+ B cells - % of B cells IMPC_IMM_173_001 | v1.0

simpleParameter

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

Unit Measured: %

Follicular B cells - % of B cells IMPC_IMM_174_001 | v1.0

simpleParameter

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

Unit Measured: %

Ly6C+ CD11b- NK cells - % of NK cells (Panel B) IMPC_IMM_175_001 | v1.0

simpleParameter

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

Unit Measured: %

Ly6C+ CD11b+ NK cells - % of NK cells (Panel B) IMPC_IMM_176_001 | v1.0

simpleParameter

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

Unit Measured: %

Ly6C+ NKT cells - % of NKT cells (Panel B) IMPC_IMM_177_001 | v1.0

simpleParameter

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

Unit Measured: %

Marginal zone B cells - % of B cells IMPC_IMM_178_001 | v1.0

simpleParameter

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

Unit Measured: %

Transitional 1 Bcells - % of B cells IMPC_IMM_179_001 | v1.0

simpleParameter

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

Unit Measured: %

Transitional 2 B cells - % of B cells IMPC_IMM_180_001 | v1.0

simpleParameter

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

Unit Measured: %

T cells (Panel A) - cell count IMPC_IMM_181_001 | v1.0

simpleParameter

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

Unit Measured: count

T cells - cell count IMPC_IMM_182_001 | v1.0

simpleParameter

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

Unit Measured: count

CD8+ T cells - cell count IMPC_IMM_183_001 | v1.0

simpleParameter

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

Unit Measured: count

Effector CD8+ T cells - cell count IMPC_IMM_184_001 | v1.0

simpleParameter

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

Unit Measured: count

Resting CD8+ T cells - cell count IMPC_IMM_185_001 | v1.0

simpleParameter

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

Unit Measured: count

Naïve CD8+ T cells - cell count IMPC_IMM_186_001 | v1.0

simpleParameter

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

Unit Measured: count

Klrg1+ CD8 T cells - cell count IMPC_IMM_187_001 | v1.0

simpleParameter

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

Unit Measured: count

CD4 T cells - cell count IMPC_IMM_188_001 | v1.0

simpleParameter

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

Unit Measured: count

CD4+ T helper cells - cell count IMPC_IMM_189_001 | v1.0

simpleParameter

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

Unit Measured: count

Effector CD4+ T helper cells - cell count IMPC_IMM_190_001 | v1.0

simpleParameter

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

Unit Measured: count

Resting CD4+ T helper cells - cell count IMPC_IMM_191_001 | v1.0

simpleParameter

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

Unit Measured: count

Klrg1+ CD4+ T helper cells - cell count IMPC_IMM_192_001 | v1.0

simpleParameter

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

Unit Measured: count

Treg cells - cell count IMPC_IMM_193_001 | v1.0

simpleParameter

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

Unit Measured: count

Effector Treg cells - cell count IMPC_IMM_194_001 | v1.0

simpleParameter

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

Unit Measured: count

Resting Treg cells - cell count IMPC_IMM_195_001 | v1.0

simpleParameter

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

Unit Measured: count

Klrg1+ Treg cells - cell count IMPC_IMM_196_001 | v1.0

simpleParameter

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

Unit Measured: count

T cells - cell count IMPC_IMM_197_001 | v1.0

simpleParameter

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

Unit Measured: count

Effector T cells - cell count IMPC_IMM_198_001 | v1.0

simpleParameter

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

Unit Measured: count

Resting T cells - cell count IMPC_IMM_199_001 | v1.0

simpleParameter

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

Unit Measured: count

Klrg1+ T cells - cell count IMPC_IMM_200_001 | v1.0

simpleParameter

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

Unit Measured: count

NKT cells (panel A) - cell count IMPC_IMM_201_001 | v1.0

simpleParameter

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

Unit Measured: count

CD4+ NKT cells - cell count IMPC_IMM_202_001 | v1.0

simpleParameter

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

Unit Measured: count

Effector CD4+ NKT cells - cell count IMPC_IMM_203_001 | v1.0

simpleParameter

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

Unit Measured: count

Resting CD4+ NKT cells - cell count IMPC_IMM_204_001 | v1.0

simpleParameter

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

Unit Measured: count

Klrg1+ CD4+ NKT cells - cell count IMPC_IMM_205_001 | v1.0

simpleParameter

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

Unit Measured: count

CD4- NKT cells - cell count IMPC_IMM_206_001 | v1.0

simpleParameter

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

Unit Measured: count

Effector CD4- NKT cells - cell count IMPC_IMM_207_001 | v1.0

simpleParameter

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

Unit Measured: count

Resting CD4- NKT cells - cell count IMPC_IMM_208_001 | v1.0

simpleParameter

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

Unit Measured: count

Klrg1+ CD4- NKT cells - cell count IMPC_IMM_209_001 | v1.0

simpleParameter

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

Unit Measured: count

NK cells (Panel A) - cell count IMPC_IMM_210_001 | v1.0

simpleParameter

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

Unit Measured: count

Effector NK cells - cell count IMPC_IMM_211_001 | v1.0

simpleParameter

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

Unit Measured: count

Resting NK cells - cell count IMPC_IMM_212_001 | v1.0

simpleParameter

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

Unit Measured: count

Klrg1+ NK cells - cell count IMPC_IMM_213_001 | v1.0

simpleParameter

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

Unit Measured: count

T cells (panel B) - cell count IMPC_IMM_214_001 | v1.0

simpleParameter

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

Unit Measured: count

NKT cells (panel B) - cell count IMPC_IMM_215_001 | v1.0

simpleParameter

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

Unit Measured: count

Ly6C+ NKT cells - cell count IMPC_IMM_216_001 | v1.0

simpleParameter

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

Unit Measured: count

NK cells (Panel B) - cell count IMPC_IMM_217_001 | v1.0

simpleParameter

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

Unit Measured: count

CD11b- NK cells - cell count IMPC_IMM_218_001 | v1.0

simpleParameter

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

Unit Measured: count

Ly6C+ CD11b- NK cells - cell count IMPC_IMM_219_001 | v1.0

simpleParameter

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

Unit Measured: count

CD11b+ NK cells - cell count IMPC_IMM_220_001 | v1.0

simpleParameter

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

Unit Measured: count

Ly6C+ CD11b+ NK cells - cell count IMPC_IMM_221_001 | v1.0

simpleParameter

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

Unit Measured: count

B cells - cell count IMPC_IMM_222_001 | v1.0

simpleParameter

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

Unit Measured: count

B1a cells - cell count IMPC_IMM_223_001 | v1.0

simpleParameter

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

Unit Measured: count

B1b cells - cell count IMPC_IMM_224_001 | v1.0

simpleParameter

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

Unit Measured: count

Follicular B cells - cell count IMPC_IMM_225_001 | v1.0

simpleParameter

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

Unit Measured: count

Marginal zone B cells - cell count IMPC_IMM_226_001 | v1.0

simpleParameter

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

Unit Measured: count

Transitional 1 B cells - cell count IMPC_IMM_227_001 | v1.0

simpleParameter

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

Unit Measured: count

Transitional 2 B cells - cell count IMPC_IMM_228_001 | v1.0

simpleParameter

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

Unit Measured: count

CD161+ B cells - cell count IMPC_IMM_229_001 | v1.0

simpleParameter

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

Unit Measured: count

Conventional DC - cell count IMPC_IMM_230_001 | v1.0

simpleParameter

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

Unit Measured: count

CD11b-low cDC - cell count IMPC_IMM_231_001 | v1.0

simpleParameter

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

Unit Measured: count

CD11b-high cDC - cell count IMPC_IMM_232_001 | v1.0

simpleParameter

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

Unit Measured: count

Plasmacytoid DC - cell count IMPC_IMM_233_001 | v1.0

simpleParameter

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

Unit Measured: count

Macrophages - cell count IMPC_IMM_234_001 | v1.0

simpleParameter

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

Unit Measured: count

Monocytes - cell count IMPC_IMM_235_001 | v1.0

simpleParameter

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

Unit Measured: count

Granulocytes - cell count IMPC_IMM_236_001 | v1.0

simpleParameter

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

Unit Measured: count

Eosinophils - cell count IMPC_IMM_237_001 | v1.0

simpleParameter

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

Unit Measured: count

Panel A anti-CD5 clone IMPC_IMM_238_001 | v1.0

procedureMetadata

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

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Panel A anti-CD5 fluorochrome IMPC_IMM_239_001 | v1.0

procedureMetadata

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

Options: BV421, eF450,

Panel A anti-CD5 RRID IMPC_IMM_240_001 | v1.0

procedureMetadata

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

Options: AB_2737758, AB_1603250,

Panel A anti-CD4 clone IMPC_IMM_241_001 | v1.0

procedureMetadata

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

Options: RM4-5, GK1.5,

Panel A anti-CD4 fluorochrome IMPC_IMM_242_001 | v1.0

procedureMetadata

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

Options: FITC, PO,

Panel A anti-CD4 RRID IMPC_IMM_243_001 | v1.0

procedureMetadata

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

Options: AB_394583, AB_1474250, AB_396633,

Panel A anti-CD44 clone IMPC_IMM_244_001 | v1.0

procedureMetadata

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

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Panel A anti-CD44 fluorochrome IMPC_IMM_245_001 | v1.0

procedureMetadata

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

Options: PE, BV650,

Panel A anti-CD44 RRID IMPC_IMM_246_001 | v1.0

procedureMetadata

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

Options: AB_394649, AB_2562600, AB_10895375,

Panel A anti-CD8a clone IMPC_IMM_247_001 | v1.0

procedureMetadata

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

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Panel A anti-CD8a fluorochrome IMPC_IMM_248_001 | v1.0

procedureMetadata

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

Options: PE-CF594, APCeF780,

Panel A anti-CD8a RRID IMPC_IMM_249_001 | v1.0

procedureMetadata

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

Options: AB_11152075, AB_1272185,

Panel A anti-CD25 clone IMPC_IMM_250_001 | v1.0

procedureMetadata

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

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Panel A anti-CD25 fluorochrome IMPC_IMM_251_001 | v1.0

procedureMetadata

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

Options: PE-Cy7, APC,

Panel A anti-CD25 RRID IMPC_IMM_252_001 | v1.0

procedureMetadata

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

Options: AB_394509, AB_398623, AB_10562035,

Panel A anti-CD161 clone IMPC_IMM_253_001 | v1.0

procedureMetadata

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

---

Panel A anti-CD161 fluorochrome IMPC_IMM_254_001 | v1.0

procedureMetadata

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

Options: APC, PE, PE-Cy7,

Panel A anti-CD161 RRID IMPC_IMM_255_001 | v1.0

procedureMetadata

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

Options: AB_398463, AB_394677, AB_394507,

Panel A anti-CD62L clone IMPC_IMM_256_001 | v1.0

procedureMetadata

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

---

Panel A anti-CD62L fluorochrome IMPC_IMM_257_001 | v1.0

procedureMetadata

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

Options: APC-Cy7, PE-Cy7,

Panel A anti-CD62L RRID IMPC_IMM_258_001 | v1.0

procedureMetadata

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

Options: AB_10611861, AB_469633,

Panel A Live/Dead stain IMPC_IMM_259_001 | v1.0

procedureMetadata

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

Options: PI, Aqua, Sytox Blue,

Panel A additional maker 1 name IMPC_IMM_260_001 | v1.0

procedureMetadata

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

---

Panel A additional marker 1 clone IMPC_IMM_261_001 | v1.0

procedureMetadata

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

---

Panel A additional marker 1 fluorochrome IMPC_IMM_262_001 | v1.0

procedureMetadata

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

---

Panel A additional marker 1 RRID IMPC_IMM_263_001 | v1.0

procedureMetadata

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

Panel A additional maker 2 name IMPC_IMM_264_001 | v1.0

procedureMetadata

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

---

Panel A additional marker 2 clone IMPC_IMM_265_001 | v1.0

procedureMetadata

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

---

Panel A additional marker 2 fluorochrome IMPC_IMM_266_001 | v1.0

procedureMetadata

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

---

Panel A additional marker 2 RRID IMPC_IMM_267_001 | v1.0

procedureMetadata

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

---

Panel A additional maker 3 name IMPC_IMM_268_001 | v1.0

procedureMetadata

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

---

Panel A additional marker 3 clone IMPC_IMM_269_001 | v1.0

procedureMetadata

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

---

Panel A additional marker 3 fluorochrome IMPC_IMM_270_001 | v1.0

procedureMetadata

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

---

Panel A additional marker 3 RRID IMPC_IMM_271_001 | v1.0

procedureMetadata

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

---

Panel A additional maker 4 name IMPC_IMM_272_001 | v1.0

procedureMetadata

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

---

Panel A additional marker 4 clone IMPC_IMM_273_001 | v1.0

procedureMetadata

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

---

Panel A additional marker 4 fluorochrome IMPC_IMM_274_001 | v1.0

procedureMetadata

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

---

Panel A additional marker 4 RRID IMPC_IMM_275_001 | v1.0

procedureMetadata

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

---

Panel A additional maker 5 name IMPC_IMM_276_001 | v1.0

procedureMetadata

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

---

Panel A additional marker 5 clone IMPC_IMM_277_001 | v1.0

procedureMetadata

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

---

Panel A additional marker 5 fluorochrome IMPC_IMM_278_001 | v1.0

procedureMetadata

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

---

Panel A additional marker 5 RRID IMPC_IMM_279_001 | v1.0

procedureMetadata

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

---

Panel B anti-CD5 clone IMPC_IMM_280_001 | v1.0

procedureMetadata

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

---

Panel B anti-CD5 fluorochrome IMPC_IMM_281_001 | v1.0

procedureMetadata

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

---

Panel B anti-CD5 RRID IMPC_IMM_282_001 | v1.0

procedureMetadata

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

---

Panel B anti-Ly6G clone IMPC_IMM_283_001 | v1.0

procedureMetadata

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

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Panel B anti-Ly6G fluorochrome IMPC_IMM_284_001 | v1.0

procedureMetadata

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

Options: BV421, BV785,

Panel B anti-Ly6G RRID IMPC_IMM_285_001 | v1.0

procedureMetadata

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

Options: AB_2737756, AB_2566317,

Panel B anti-CD19 clone IMPC_IMM_286_001 | v1.0

procedureMetadata

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

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Panel B anti-CD19 fluorochrome IMPC_IMM_287_001 | v1.0

procedureMetadata

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

Options: BV510, PE-Cy7,

Panel B anti-CD19 RRID IMPC_IMM_288_001 | v1.0

procedureMetadata

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

Options: AB_2737915, AB_394495,

Panel B anti-Ly6C clone IMPC_IMM_289_001 | v1.0

procedureMetadata

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

Options: AL-21, HK1.4,

Panel B anti-Ly6c fluorochrome IMPC_IMM_290_001 | v1.0

procedureMetadata

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

Options: FITC, PerCP Cy5.5,

Panel B anti-Ly6c RRID IMPC_IMM_291_001 | v1.0

procedureMetadata

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

Options: AB_394628, AB_2723343,

Panel B anti-CD21/35 clone IMPC_IMM_292_001 | v1.0

procedureMetadata

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

---

Panel B anti-CD21/35 fluorochrome IMPC_IMM_293_001 | v1.0

procedureMetadata

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

Options: PE, BV605,

Panel B anti-CD21/35 RRID IMPC_IMM_294_001 | v1.0

procedureMetadata

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

Options: AB_394532, AB_2738048,

Panel B anti-CD11b clone IMPC_IMM_295_001 | v1.0

procedureMetadata

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

---

Panel B anti-CD11b fluorochrome IMPC_IMM_296_001 | v1.0

procedureMetadata

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

Options: PE-CF594, PerCP-Cy5.5,

Panel B anti-CD11b RRID IMPC_IMM_297_001 | v1.0

procedureMetadata

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

Options: AB_11154216, AB_2033995,

Panel B anti-CD11c clone IMPC_IMM_298_001 | v1.0

procedureMetadata

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

---

Panel B anti-CD11c fluorochrome IMPC_IMM_299_001 | v1.0

procedureMetadata

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

Options: PE-Cy7, APC-Cy7,

Panel B anti-CD11c RRID IMPC_IMM_300_001 | v1.0

procedureMetadata

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

Options: AB_647251, AB_10611727,

Panel B anti-CD161 clone IMPC_IMM_301_001 | v1.0

procedureMetadata

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

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Panel B anti-CD161 fluorochrome IMPC_IMM_302_001 | v1.0

procedureMetadata

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

Panel B anti-CD161 RRID IMPC_IMM_303_001 | v1.0

procedureMetadata

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

---

Panel B anti-MHCII clone IMPC_IMM_304_001 | v1.0

procedureMetadata

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

---

Panel B anti-MHCII fluorochrome IMPC_IMM_305_001 | v1.0

procedureMetadata

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

Options: APC-eFluor(R) 780, BV650, APC-Cy7,

Panel B anti-MHCII RRID IMPC_IMM_306_001 | v1.0

procedureMetadata

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

Options: AB_1548783, AB_2565975, AB_2069377,

Panel B Live/Dead stain IMPC_IMM_307_001 | v1.0

procedureMetadata

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

Options: PI, Aqua, Sytox Blue,

Panel B additional maker 1 name IMPC_IMM_308_001 | v1.0

procedureMetadata

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

---

Panel B additional marker 1 clone IMPC_IMM_309_001 | v1.0

procedureMetadata

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

---

Panel B additional marker 1 fluorochrome IMPC_IMM_310_001 | v1.0

procedureMetadata

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

---

Panel B additional marker 1 RRID IMPC_IMM_311_001 | v1.0

procedureMetadata

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

---

Panel B additional maker 2 name IMPC_IMM_312_001 | v1.0

procedureMetadata

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

---

Panel B additional marker 2 clone IMPC_IMM_313_001 | v1.0

procedureMetadata

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

---

Panel B additional marker 2 fluorochrome IMPC_IMM_314_001 | v1.0

procedureMetadata

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

---

Panel B additional marker 2 RRID IMPC_IMM_315_001 | v1.0

procedureMetadata

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

---

Panel B additional maker 3 name IMPC_IMM_316_001 | v1.0

procedureMetadata

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

---

Panel B additional marker 3 clone IMPC_IMM_317_001 | v1.0

procedureMetadata

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

---

Panel B additional marker 3 fluorochrome IMPC_IMM_318_001 | v1.0

procedureMetadata

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

Panel B additional marker 3 RRID IMPC_IMM_319_001 | v1.0

procedureMetadata

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

---

Panel B additional maker 4 name IMPC_IMM_320_001 | v1.0

procedureMetadata

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

---

Panel B additional marker 4 clone IMPC_IMM_321_001 | v1.0

procedureMetadata

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

---

Panel B additional marker 4 fluorochrome IMPC_IMM_322_001 | v1.0

procedureMetadata

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

---

Panel B additional marker 4 RRID IMPC_IMM_323_001 | v1.0

procedureMetadata

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

---

Panel B additional maker 5 name IMPC_IMM_324_001 | v1.0

procedureMetadata

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

---

Panel B additional marker 5 clone IMPC_IMM_325_001 | v1.0

procedureMetadata

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

---

Panel B additional marker 5 fluorochrome IMPC_IMM_326_001 | v1.0

procedureMetadata

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

---

Panel B additional marker 5 RRID IMPC_IMM_327_001 | v1.0

procedureMetadata

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

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Analysis results file IMPC_IMM_328_001 | v1.0

mediaParameter

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

Description: A csv file with the analysis results for the mutant line

---