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

- 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
  - Equipment for manual dissociation
- Cell counter equipment:
  - Orflo Moxi-Z Cell counter
  - Coulter Vicell XR OR Life Technologies Attune® Flow Cytometer
  - 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)
- 70-80µM Nytex
- Cell counter recipients (i.e., slides/cassettes/etc. for cell counter)
- *(if sample processing delayed)* RPMI 1640
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 −20°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\(^{2+}\).

1. **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.

2. **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

3. **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)

4. **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

5. **RBC Lysis buffer:** Prepare a 1X solution in ddH\(_2\)O from 10X stock lysis buffer.

6. **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 mixture.
  - 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 20,000xg and 8ºC 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

<table>
<thead>
<tr>
<th>Type</th>
<th>Antibody (Marker)</th>
<th>Clone</th>
<th>Fluorochrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Required</td>
<td>CD5</td>
<td>53-7.3</td>
<td>BV421</td>
</tr>
<tr>
<td>Required</td>
<td>CD4</td>
<td>RM4-5</td>
<td>FITC</td>
</tr>
<tr>
<td>Required</td>
<td>CD44</td>
<td>IM7</td>
<td>PE</td>
</tr>
<tr>
<td>Required</td>
<td>CD8a</td>
<td>53-6.7</td>
<td>PE-CF594</td>
</tr>
<tr>
<td>Required</td>
<td>CD25</td>
<td>PC61</td>
<td>PE-Cy7</td>
</tr>
<tr>
<td>Required</td>
<td>CD161</td>
<td>PK136</td>
<td>APC</td>
</tr>
<tr>
<td>Required</td>
<td>CD62L</td>
<td>MEL-14</td>
<td>APC-Cy7</td>
</tr>
<tr>
<td>Required</td>
<td>Live/Dead</td>
<td>-</td>
<td>SytoxBlue</td>
</tr>
<tr>
<td>Type</td>
<td>Antibody (Marker)</td>
<td>Clone</td>
<td>Fluorochrome</td>
</tr>
<tr>
<td>----------</td>
<td>-------------------</td>
<td>--------</td>
<td>--------------</td>
</tr>
<tr>
<td>Optional</td>
<td>CD3e</td>
<td>145-2C11</td>
<td></td>
</tr>
<tr>
<td>Optional</td>
<td>CD24</td>
<td>M1/69</td>
<td></td>
</tr>
<tr>
<td>Optional</td>
<td>CD27</td>
<td>LG.3A10</td>
<td></td>
</tr>
<tr>
<td>Optional</td>
<td>CD357/GITR</td>
<td>DTA-1</td>
<td></td>
</tr>
<tr>
<td>Optional</td>
<td>CD45</td>
<td>30-F11</td>
<td></td>
</tr>
<tr>
<td>Optional</td>
<td>KLRG1</td>
<td>2F1</td>
<td></td>
</tr>
<tr>
<td>Optional</td>
<td>Ly6c</td>
<td>AL-21</td>
<td></td>
</tr>
<tr>
<td>Optional</td>
<td>TCRd</td>
<td>GL-3</td>
<td></td>
</tr>
</tbody>
</table>

### Panel B

<table>
<thead>
<tr>
<th>Type</th>
<th>Antibody (Marker)</th>
<th>Clone</th>
<th>Fluorochrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Required</td>
<td>CD5</td>
<td>53-7.3</td>
<td>BV421</td>
</tr>
<tr>
<td>Required</td>
<td>Ly6G</td>
<td>1A3</td>
<td>BV421</td>
</tr>
<tr>
<td>Required</td>
<td>CD19</td>
<td>1D3</td>
<td>BV510</td>
</tr>
<tr>
<td>Required</td>
<td>Ly6C</td>
<td>AL-21</td>
<td>FITC</td>
</tr>
<tr>
<td>Required</td>
<td>CD21/CD35</td>
<td>7G6</td>
<td>PE</td>
</tr>
<tr>
<td>Required</td>
<td>CD11b</td>
<td>M1/70</td>
<td>PE-CF594</td>
</tr>
</tbody>
</table>
- **Required**
  - CD11c
  - HL3
  - PE-Cy7
  - CD161
  - PK136
  - APC
  - MHCII
  - M5/114.15.2
  - APC-Cy7 or A700
  - Live/Dead
  - SytoxBlue

- **Optional**
  - CD23
  - B3B4
  - CD27
  - LG3.A10
  - CD43
  - S7
  - CD44
  - CD45
  - 30-F11
  - CD317
  - 927
  - F4/80
  - BM8
  - IgD
  - 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

- **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 -20°C, do not freeze-thaw stock)
• Collagenase D: 600 Mandl Units (from 30 U/µl stock in enzyme buffer stored in single experiment aliquots at -20°C, 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 Ca²⁺ and Mg²⁺ (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 37°C 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 10°C. 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 10°C, 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

CD45 not included? Omit this step

TCRδ not included? Go straight to page 2

Continue on page 2
2. NK cells and further gating
3. NKT cells

![Diagram of NKT cell distributions and markers](image-url)
4. Tregs and T helper cells

**Panel A. Page 4.**

**or**

GITR included  

GITR not included
5. CD8 T cells and notes

1. If there is no CD45 in the panel, omit this step.
2. Approximately 50% of y6 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 KIrG1+ 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 y6 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 MIBI 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

- Use F4/80 to identify macrophages (~1% of live lymphocytes) or skip

- Go to 5

- CD161

- Go to 4

- CD161

- CD19

- Go to 3

- CD5/Ly6G

- CD19

- Go to 2

- CD19

- CD5/Ly6G

- Use CD5 if separate channels
2A. B cells – core panel only (CD21/35 & CD5/Ly6G)

CD19+

CD161+

B1a

CD161−
Panel B. Page 2B.

2B. B cells – core panel with optional CD23

- CD19+ CD161+
- CD161-
- MZ B
- FO B
- Non-MZ/FO
- Non-MZ/FO
- B-1a
- Transitional/B-1b/PB
Panel B. Page 2C.

2C. B cells – core panel with optional CD23 & CD43

- CD19+ CD161+
- CD161-
- CD43+
- CD43-

T-1, T-2
Non-MZ/FO
MZ B
FO B
Non-MZ/FO
B-1a
B-1b/ PB
3. T cells and NK T cells

Use CDS if separate channels
4. NK cells
Parameters and Metadata

**Spleen weight** IMPC_IMM_001_002 | v2.0

simpleParameter


Unit Measured: g

---

**Live leukocytes (Panel A) - % of total events** IMPC_IMM_002_002 | v2.0
T cells (Panel A) - % of live leukocytes (Panel A)  IMPC_IMM_0
03_002 | v2.0

NKT cells (panel A) - % of live leukocytes (Panel A)  IMPC_IMM_M_004_002 | v2.0

NK cells (Panel A) - % of live leukocytes (Panel A)  IMPC_IMM_005_002 | v2.0
CD4 T cells - % of live leukocytes (Panel A) IMPC_IMM_007_002
| v2.0
simpleParameter

CD8+ T cells - % of live leukocytes (Panel A) IMPC_IMM_008_002
| v2.0
simpleParameter

CD4+ NKT cells - % of live leukocytes (Panel A) IMPC_IMM_01
_002 | v2.0
simpleParameter
CD4- NKT cells - % of live leukocytes (Panel A) IMPC_IMM_013_002 | v2.0
simpleParameter

Treg cells - % of live leukocytes (Panel A) IMPC_IMM_014_002 | v2.0
simpleParameter

CD4+ T helper cells - % of live leukocytes (Panel A) IMPC_IMM_015_002 | v2.0
simpleParameter
Total events (Panel A) IMPC_IMM_026_002 | v2.0

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

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

Unit Measured: %

Effector CD8+ T cells - % of live leukocytes (Panel A) IMPC_IMM_032_002 | v2.0

Unit Measured: %

Naïve CD8+ T cells - % of live leukocytes (Panel A) IMPC_IMM_033_002 | v2.0

Unit Measured: %
**Resting CD8+ T cells - % of live leukocytes (Panel A)**

**MM_034_002 | v2.0**

**Unit Measured:** %

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

---

**Effector CD4+ NKT cells - % of live leukocytes (Panel A)**

**PC_IMM_040_002 | v2.0**

**Unit Measured:** %

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

---

**Resting CD4+ NKT cells - % of live leukocytes (Panel A)**

**PC_IMM_041_002 | v2.0**

**Unit Measured:** %

**Req. Analysis:** false  
**Req. Upload:** true  
**Is Annotated:** false
**Effector CD4- NKT cells - % of live leukocytes (Panel A)**

PC_IMM_046_002 | v2.0

simpleParameter

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

**Unit Measured:** %

---

**Resting CD4- NKT cells - % of live leukocytes (Panel A)**

C_IMM_047_002 | v2.0

simpleParameter

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

**Unit Measured:** %

---

**Live leukocytes (Panel B) - % of total events (Panel B)**

IMPC_IMM_049_002 | v2.0

simpleParameter

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

**Unit Measured:** %
Granulocytes - % of live leukocytes (Panel B)  IMPC_IMM_050_0
02 | v2.0


Unit Measured: %

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


Unit Measured: %

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


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

Unit Measured: %

Ly6C+ CD11b- NK cells - % of live leukocytes (Panel B) IMPC_IMM_054_002 | v2.0

Unit Measured: %

Ly6C+ CD11b+ NK cells - % of live leukocytes (Panel B) IMPC_IMM_055_002 | v2.0

Unit Measured: %
CD11b- NK cells - % of live leukocytes (Panel B) IMPC_IMM_0
56_002 | v2.0
simpleParameter

Unit Measured: %

CD11b+ NK cells - % of live leukocytes (Panel B) IMPC_IMM_0
57_002 | v2.0
simpleParameter

Unit Measured: %

NKT cells (panel B) - % of live leukocytes (Panel B) IMPC_IMM_0
M_058_002 | v2.0
simpleParameter

Unit Measured: %
Ly6C+ NKT cells - % of live leukocytes (Panel B) IMPC_IMM_0

59_002 | v2.0

simpleParameter


Unit Measured: %

-------------------------------------------------------------------------------------------------------------------------------------

T cells (panel B) - % of live leukocytes (Panel B) IMPC_IMM_06

1_002 | v2.0

simpleParameter


Unit Measured: %

-------------------------------------------------------------------------------------------------------------------------------------

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

simpleParameter


Unit Measured: %

-------------------------------------------------------------------------------------------------------------------------------------

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

0
Marginal zone B cells - % of B cells (Panel B)  IMPC_IMM_071_0
02 | v2.0

Conventional DC - % of live leukocytes (Panel B)  IMPC_IMM_0
72_002 | v2.0

Plasmacytoid DC- % of live leukocytes (Panel B)  IMPC_IMM_0
74_002 | v2.0
Macrophages- % of live leukocytes (Panel B) IMPC_IMM_075_0

02 | v2.0

Equipment name IMPC_IMM_077_002 | v2.0

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

Equipment manufacturer IMPC_IMM_078_002 | v2.0
**Options**: BD Biosciences, Beckman Coulter, IntelliCyt, Cytek,

**Equipment model** IMPC_IMM_079_002 | v2.0

**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

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

**Anesthesia** IMPC_IMM_081_002 | v2.0

**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


Cell digestion agent  IMPC_IMM_083_002 | v2.0

procedureMetadata


Cell digestion agent manufacturer  IMPC_IMM_084_002 | v2.0

procedureMetadata


Cell digestion agent catalog number  IMPC_IMM_085_002 | v2.0

procedureMetadata

Cell counting performed IMPC_IMM_086_002 | v2.0

Options: Cellometer Auto T4, Cellometer,

Cell counting equipment manufacturer IMPC_IMM_087_002 | v2.0

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

Cell counting equipment model IMPC_IMM_088_002 | v2.0

Cell counting equipment name IMPC_IMM_089_002 | v2.0
**Cell lysis buffer manufacturer**  IMPC_IMM_090_002 | v2.0

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

**Cell lysis buffer catalog number**  IMPC_IMM_091_002 | v2.0

**Date and time of sacrifice**  IMPC_IMM_092_002 | v2.0

**Date and time of sample preparation**  IMPC_IMM_093_002 | v2.0
Sample storage temperature until analysis (in Celsius) IMP
C_IMM_094_002 | v2.0

**Procedure Metadata**

**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

**Procedure Metadata**

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

---

**Balanced salt solution type** IMPC_IMM_096_002 | v2.0

**Procedure Metadata**

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

**Options:** HBSS, PBS, KDS BSS,

---

**Balanced salt solution manufacturer** IMPC_IMM_097_002 | v2.0
Balanced salt solution catalog number IMPC_IMM_098_002 | v2.0

RPMI manufacturer IMPC_IMM_099_002 | v2.0

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

RPMI catalog number IMPC_IMM_100_002 | v2.0

DNAse I manufacturer IMPC_IMM_101_002 | v2.0
Procedure Metadata

**DNAse I catalog number** IMPC_IMM_102_002 | v2.0

**Dead cell exclusion dye** IMPC_IMM_103_002 | v2.0

**Dead cell exclusion dye manufacturer** IMPC_IMM_104_002 | v2.0
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

Options: 423106, D9542, home brew, R37606, S-34860, S11348, S34857, P4170, L34966, 13-0868-T500, T8154-100ML,

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

Panel A FCS file(s) IMPC_IMM_107_002 | v2.0
Panel B FCS file(s) IMPC_IMM_108_002 | v2.0


Automated analysis IMPC_IMM_109_002 | v2.0


Collection buffer manufacturer IMPC_IMM_110_002 | v2.0


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

Collection buffer catalog number number IMPC_IMM_111_002 | v2.0


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


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

--------------------------------------------------------------------------------------------------

**Total spleen leukocyte count** IMPC_IMM_116_001 | v1.0

simpleParameter


--------------------------------------------------------------------------------------------------

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

simpleParameter


--------------------------------------------------------------------------------------------------

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

simpleParameter

**Effector NK cells - % of live leukocytes (Panel A)** IMPC_IMM_1
19_001 | v1.0
simpleParameter

**Unit Measured:** %

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

**Unit Measured:** %

**Effector T cells - % of live leukocytes (Panel A)** IMPC_IMM_12
1_001 | v1.0
simpleParameter

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

Unit Measured: %

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

Unit Measured: %

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

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

Unit Measured: %

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

Unit Measured: %

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

Unit Measured: %
**Klr1+ T cells - % of live leukocytes (Panel A)**

IMPC_IMM_128_001 | v1.0

Unit Measured: %

---

**Resting NK cells - % of live leukocytes (Panel A)**

IMPC_IMM_129_001 | v1.0

Unit Measured: %

---

**Resting Treg cells - % of live leukocytes (Panel A)**

IMPC_IMM_130_001 | v1.0

Unit Measured: %
Resting T cells - % of live leukocytes (Panel A) IMPC_IMM_13
1_001 | v1.0

simpleParameter

Unit Measured: %

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

simpleParameter

Unit Measured: %

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

simpleParameter

Unit Measured: %

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

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

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

CD4+ T helper cells - % of CD4 T cells IMPC_IMM_137_001 | v1.0
simpleParameter
CD8+ T cells - % of T cells | IMPC_IMM_138_001 | v1.0

Simple parameter

Unit Measured: %

-----------------------------------------------

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

Simple parameter

Unit Measured: %

-----------------------------------------------

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

Simple parameter

Unit Measured: %
Effector CD4+ T helper cells - % of CD4+ T helper cells
C_IMM_141_001 | v1.0

Unit Measured: %

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

Unit Measured: %

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

Unit Measured: %
**Effector Treg cells - % of Treg cells** IMPC_IMM_144_001 | v1.0

- **simpleParameter**
- **Unit Measured:** %

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

- **simpleParameter**
- **Unit Measured:** %

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

- **simpleParameter**
- **Unit Measured:** %
**Klrg1+ CD4+ NKT cells - % of CD4+ NKT cells**  
IMPC_IMM_147_001 | v1.0

simpleParameter

**Unit Measured:** %

---

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

simpleParameter

**Unit Measured:** %

---

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

simpleParameter

**Unit Measured:** %

---

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

simpleParameter

**Unit Measured:** %
Klrg1+ Treg cells - % of Treg cells  IMPC_IMM_151_001 | v1.0

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

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

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

simpleParameter


Unit Measured: %

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

simpleParameter


Unit Measured: %

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

simpleParameter

Unit Measured: %

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

simpleParameter


Unit Measured: %

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

simpleParameter


Unit Measured: %

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

simpleParameter


Unit Measured: %
Resting T cells - % of T cells IMPC_IMM_160_001 | v1.0

Unit Measured: %

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

Unit Measured: %

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

Unit Measured: %

FSC/SSC Singlets (Panel B) IMPC_IMM_163_001 | v1.0
B1a cells - % of B cells (Panel B) IMPC_IMM_164_001 | v1.0

Unit Measured: %

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

Unit Measured: %

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

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

Unit Measured: %

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

Unit Measured: %

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

Unit Measured: %
**CD161+ B cells - % of B cells**  IMPC_IMM_173_001 | v1.0


Unit Measured: %

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


Unit Measured: %

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


Unit Measured: %

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


Unit Measured: %
Ly6C+ NKT cells - % of NKT cells (Panel B) IMPC_IMM_177_001

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

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

**Derivation:** unimplemented()

---

**T cells - cell count** IMPC_IMM_182_001 | v1.0

```
simpleParameter
```

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

Unit Measured: count
Derivation: unimplemented()

---

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


Unit Measured: count

Derivation: unimplemented()

---

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


Unit Measured: count

Derivation: unimplemented()

---

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


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

simpleParameter


Unit Measured: count

Derivation: unimplemented()

---

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

simpleParameter


Unit Measured: count

Derivation: unimplemented()

---

CD4 T cells - cell count  IMPC_IMM_188_001 | v1.0

simpleParameter

**Unit Measured:** count

**Derivation:** unimplemented()

---

**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

**Derivation:** unimplemented()

---

**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

**Derivation:** unimplemented()

---

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

simpleParameter
Klrng1+ CD4+ T helper cells - cell count IMPC_IMM_192_001 | v1.0
unimplemented()

Treg cells - cell count IMPC_IMM_193_001 | v1.0
unimplemented()

Effector Treg cells - cell count IMPC_IMM_194_001 | v1.0
unimplemented()
**Resting Treg cells - cell count**  IMPC_IMM_195_001 | v1.0

simpleParameter

**Klr g1+ Treg cells - cell count**  IMPC_IMM_196_001 | v1.0

simpleParameter

**T cells - cell count**  IMPC_IMM_197_001 | v1.0

simpleParameter
Effector T cells - cell count IMPC_IMM_198_001 | v1.0

Resting T cells - cell count IMPC_IMM_199_001 | v1.0

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


Unit Measured: count

Derivation: unimplemented()

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

simpleParameter


Unit Measured: count

Derivation: unimplemented()

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

simpleParameter


Unit Measured: count

Derivation: unimplemented()
Effector CD4+ NKT cells - cell count

- **Simple Parameter**
- **Req. Analysis**: false
- **Req. Upload**: false
- **Is Annotated**: false
- **Unit Measured**: count
- **Derivation**: unimplemented()

Resting CD4+ NKT cells - cell count

- **Simple Parameter**
- **Req. Analysis**: false
- **Req. Upload**: false
- **Is Annotated**: false
- **Unit Measured**: count
- **Derivation**: unimplemented()

KlrG1+ CD4+ NKT cells - cell count

- **Simple Parameter**
- **Req. Analysis**: false
- **Req. Upload**: false
- **Is Annotated**: false
- **Unit Measured**: count
- **Derivation**: unimplemented()
**CD4- NKT cells - cell count** IMPC_IMM_206_001 | v1.0

*simpleParameter*

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

**Unit Measured:** count

**Derivation:** unimplemented()

---

**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

**Derivation:** unimplemented()

---

**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

**Derivation:** unimplemented()
**Klrq1+ CD4- NKT cells - cell count** IMPC_IMM_209_001 | v1.0

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

**Unit Measured:** count

**Derivation:** unimplemented()

---

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

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

**Unit Measured:** count

**Derivation:** unimplemented()

---

**Effector NK cells - cell count** IMPC_IMM_211_001 | v1.0

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

**Unit Measured:** count

**Derivation:** unimplemented()
Resting NK cells - cell count  IMPC_IMM_212_001 | v1.0

simpleParameter


Unit Measured: count

Derivation: unimplemented()

---

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

simpleParameter


Unit Measured: count

Derivation: unimplemented()

---

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

simpleParameter


Unit Measured: count

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

simpleParameter


Unit Measured: count

Derivation: unimplemented()

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

simpleParameter


Unit Measured: count

Derivation: unimplemented()

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

simpleParameter


Unit Measured: count

Derivation: unimplemented()
**CD11b- NK cells - cell count** IMPC_IMM_218_001 | v1.0

*simpleParameter*

**Unit Measured:** count

**Derivation:** unimplemented()

---

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

*simpleParameter*

**Unit Measured:** count

**Derivation:** unimplemented()

---

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

*simpleParameter*

**Unit Measured:** count

**Derivation:** unimplemented()
Ly6C+ CD11b+ NK cells - cell count  IMPC_IMM_221_001 | v1.0

simpleParameter


Unit Measured: count

Derivation: unimplemented()

B cells - cell count  IMPC_IMM_222_001 | v1.0

simpleParameter


Unit Measured: count

Derivation: unimplemented()

B1a cells - cell count  IMPC_IMM_223_001 | v1.0

simpleParameter


Unit Measured: count

Derivation: unimplemented()
B1b cells - cell count  IMPC_IMM_224_001 | v1.0
simpleParameter


Unit Measured: count
Derivation: unimplemented()

Follicular B cells - cell count  IMPC_IMM_225_001 | v1.0
simpleParameter


Unit Measured: count
Derivation: unimplemented()

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


Unit Measured: count
Derivation: unimplemented()
**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

**Derivation:** unimplemented()

**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

**Derivation:** unimplemented()

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

*simpleParameter*

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

**Unit Measured:** count

**Derivation:** unimplemented()
**Conventional DC - cell count** IMPC_IMM_230_001 | v1.0

*simpleParameter*

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

Unit Measured: count

Derivation: unimplemented()

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

*simpleParameter*

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

Unit Measured: count

Derivation: unimplemented()

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

*simpleParameter*

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

Unit Measured: count

Derivation: unimplemented()
Plasmacytoid DC - cell count IMPC_IMM_233_001 | v1.0

simpleParameter


Unit Measured: count

Derivation: unimplemented()

Macrophages - cell count IMPC_IMM_234_001 | v1.0

simpleParameter


Unit Measured: count

Derivation: unimplemented()

Monocytes - cell count IMPC_IMM_235_001 | v1.0

simpleParameter


Unit Measured: count
Granulocytes - cell count  IMPC_IMM_236_001 | v1.0


Unit Measured: count

Derivation: unimplemented()

Eosinophils - cell count  IMPC_IMM_237_001 | v1.0


Unit Measured: count

Derivation: unimplemented()

Panel A anti-CD5 clone  IMPC_IMM_238_001 | v1.0


procedureMetadata
Panel A anti-CD5 fluorochrome IMPC_IMM_239_001 | v1.0
procedureMetadata


Options: BV421, eF450, PE-Gr-A,

Panel A anti-CD5 RRID IMPC_IMM_240_001 | v1.0
procedureMetadata


Options: AB_2737758, AB_1603250,

Panel A anti-CD4 clone IMPC_IMM_241_001 | v1.0
procedureMetadata


Options: RM4-5, GK1.5,

Panel A anti-CD4 fluorochrome IMPC_IMM_242_001 | v1.0
procedureMetadata
Options: FITC, PO, PE-CF594,

Panel A anti-CD4 RRID IMPC_IMM_243_001 | v1.0

Options: AB_394583, AB_1474250, AB_396633,

Panel A anti-CD44 clone IMPC_IMM_244_001 | v1.0

Options: PE, BV650, PE-Cy7,
Panel A anti-CD44 RRID IMPC_IMM_246_001 | v1.0

procedureMetadata


Options: AB_394649, AB_2562600, AB_10895375,

--------------------------------------------------------------------------------------------------------------------------

Panel A anti-CD8a clone IMPC_IMM_247_001 | v1.0

procedureMetadata


--------------------------------------------------------------------------------------------------------------------------

Panel A anti-CD8a fluorochrome IMPC_IMM_248_001 | v1.0

procedureMetadata


Options: PE-CF594, APCeF780, eFluor 450,

--------------------------------------------------------------------------------------------------------------------------

Panel A anti-CD8a RRID IMPC_IMM_249_001 | v1.0

procedureMetadata

Panel A anti-CD25 clone  IMPC_IMM_250_001 | v1.0

Options: AB_11152075, AB_1272185,
Panel A anti-CD161 clone IMPC_IMM_253_001 | v1.0
procedureMetadata


Panel A anti-CD161 fluorochrome IMPC_IMM_254_001 | v1.0
procedureMetadata


Options: APC, PE, PE-Cy7, eFluor 780,

Panel A anti-CD161 RRID IMPC_IMM_255_001 | v1.0
procedureMetadata


Options: AB_398463, AB_394677, AB_394507,

Panel A anti-CD62L clone IMPC_IMM_256_001 | v1.0
procedureMetadata

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, APC,

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

**Panel A additional marker 1 clone** IMPC_IMM_261_001 | v1.0

procedureMetadata

**Panel A additional marker 1 fluorochrome** IMPC_IMM_262_001 | v1.0

procedureMetadata

**Panel A additional marker 1 RRID** IMPC_IMM_263_001 | v1.0
Panel A additional marker 2 name  IMPC_IMM_264_001 | v1.0

procedureMetadata


Options: CD45, TCRd,

Panel A additional marker 2 clone  IMPC_IMM_265_001 | v1.0

procedureMetadata


Options: 30-F11, GL3,

Panel A additional marker 2 fluorochrome  IMPC_IMM_266_001 | v1.0

procedureMetadata


Options: BV785, BV711,
Panel A additional marker 2 RRID IMPC_IMM_267_001 | v1.0

procedureMetadata


Panel A additional marker 3 name IMPC_IMM_268_001 | v1.0

procedureMetadata


Options: CD3, KLRG1,

Panel A additional marker 3 clone IMPC_IMM_269_001 | v1.0

procedureMetadata


Options: ebio500A2, 2F1,

Panel A additional marker 3 fluorochrome IMPC_IMM_270_001 | v1.0

procedureMetadata

Panel A additional marker 3 RRID IMPC_IMM_271_001 | v1.0

Options: Af700, BV605,

Panel A additional marker 4 name IMPC_IMM_272_001 | v1.0

Options: GITR,

Panel A additional marker 4 clone IMPC_IMM_273_001 | v1.0

Options: DTA-1,
**Panel A additional marker 4 fluorochrome**  IMPC_IMM_274_001 | v1.0
procedureMetadata

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

**Options**: BV786,

**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 marker 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


Panel A additional marker 5 RRID IMPC_IMM_279_001 | v1.0


Panel B anti-CD5 clone IMPC_IMM_280_001 | v1.0


Panel B anti-CD5 fluorochrome IMPC_IMM_281_001 | v1.0


Options: BV421, eF450, PE-Gr-A,
Panel B anti-CD5 RRID IMPC_IMM_282_001 | v1.0


Panel B anti-Ly6G clone IMPC_IMM_283_001 | v1.0


Options: 1A8, RB6-8C5,

Panel B anti-Ly6G fluorochrome IMPC_IMM_284_001 | v1.0


Options: BV421, BV785, violetFluor 450,

Panel B anti-Ly6G RRID IMPC_IMM_285_001 | v1.0

Options: AB_2737756, AB_2566317,

Panel B anti-CD19 clone IMPC_IMM_286_001 | v1.0

procedureMetadata


Panel B anti-CD19 fluorochrome IMPC_IMM_287_001 | v1.0

procedureMetadata


Options: BV510, PE-Cy7, PE-CF594,

Panel B anti-CD19 RRID IMPC_IMM_288_001 | v1.0

procedureMetadata


Options: AB_2737915, AB_394495,
Panel B anti-Ly6C clone IMPC_IMM_289_001 | v1.0


Options: AL-21, HK1.4,

Panel B anti-Ly6c fluorochrome IMPC_IMM_290_001 | v1.0


Options: FITC, PerCP Cy5.5, BV785,

Panel B anti-Ly6c RRID IMPC_IMM_291_001 | v1.0


Options: AB_394628, AB_2723343,

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

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

**Procedure:***

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

**Options:** PE, BV605,

---

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

**Procedure:***

- **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

**Procedure:***

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

---

Panel B anti-CD11b fluorochrome IMPC_IMM_296_001 | v1.0

**Procedure:***

- **Req. Analysis:** false
- **Req. Upload:** false
- **Is Annotated:** false
Panel B anti-CD11b RRID IMPC_IMM_297_001 | v1.0

Panel B anti-CD11c clone IMPC_IMM_298_001 | v1.0

Panel B anti-CD11c fluorochrome IMPC_IMM_299_001 | v1.0
Panel B anti-CD11c RRID IMPC_IMM_300_001 | v1.0
procedureMetadata


Options: AB_647251, AB_10611727,

Panel B anti-CD161 clone IMPC_IMM_301_001 | v1.0
procedureMetadata


Panel B anti-CD161 fluorochrome IMPC_IMM_302_001 | v1.0
procedureMetadata


Options: APC, PE, eFluor 780,

Panel B anti-CD161 RRID IMPC_IMM_303_001 | v1.0
procedureMetadata
Panel B anti-MHCII clone IMPC_IMM_304_001 | v1.0

Options: APC-eFluor(R) 780, BV650, APC-Cy7, Alexa 700,

Panel B anti-MHCII fluorochrome IMPC_IMM_305_001 | v1.0

Options: AB_1548783, AB_2565975, AB_2069377,

Panel B anti-MHCII RRID IMPC_IMM_306_001 | v1.0

Options: AB_1548783, AB_2565975, AB_2069377,
Panel B Live/Dead stain  IMPC_IMM_307_001 | v1.0

procedureMetadata


Options: PI, Aqua, Sytox Blue,
Panel B additional marker 1 RRID IMPC_IMM_311_001 | v1.0

**procedureMetadata**


Panel B additional marker 2 name IMPC_IMM_312_001 | v1.0

**procedureMetadata**


Options: F4/80, CD317,

Panel B additional marker 2 clone IMPC_IMM_313_001 | v1.0

**procedureMetadata**


Options: BM8, eBio927,

Panel B additional marker 2 fluorochrome IMPC_IMM_314_001 | v1.0

**procedureMetadata**
Options: APC, PE-Cy7,

Panel B additional marker 2 RRID IMPC_IMM_315_001 | v1.0

Panel B additional marker 3 name IMPC_IMM_316_001 | v1.0

Panel B additional marker 3 clone IMPC_IMM_317_001 | v1.0

Options: CD45, F4/80,

Options: 30-F11, BM8,
Panel B additional marker 3 fluorochrome IMPC_IMM_318_001 | v1.0

procedureMetadata


Options: BV510, Cy5PE,
Panel B additional marker 4 fluorochrome IMPC_IMM_322_001 | v1.0

procedureMetadata


Panel B additional marker 4 RRID IMPC_IMM_323_001 | v1.0

procedureMetadata


Panel B additional marker 5 name IMPC_IMM_324_001 | v1.0

procedureMetadata


Panel B additional marker 5 clone IMPC_IMM_325_001 | v1.0

procedureMetadata

Panel B additional marker 5 fluorochrome IMPC_IMM_326_001 | v1.0

procedureMetadata


Panel B additional marker 5 RRID IMPC_IMM_327_001 | v1.0

procedureMetadata


Analysis results file IMPC_IMM_328_001 | v1.0

mediaParameter


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