# Immunophenotyping KMPCLA_IMM_001

## 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 of animals :** 3M + 3F
- **Age at test:** Week 60
- **Sex:** We would expect the results of this test to show sexual dimorphism

## 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

### Supplies

- 96-well V-bottomed plates (Falcon #353263)
- Petri dishes
- Dispensing troughs
- Extra long 10 µl 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*** 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 Mg2+, with Ca2+ (for enzyme buffer used for DNAse and Collagenase D digestions)
- PBS without Mg2+, without Ca2+ (for FACS buffer to be used in all steps subsequent to enzymatic digest)
- EDTA (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)

# 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 Ca2+ and with Mg2+, another without Ca2+ and without Mg2+.*

- **Collection buffer:**
  - (*if spleens are to be processed on the same day*) HBSS with Ca2+/Mg2+ and phenol red (Life Technologies 14170161; check if it has phenol red) ***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* Ca2+/Mg2+ ***OR***
  - HBSS 1X *without* Ca2+/Mg2+
  - EDTA 2mM
  - 2% CS (v/v)
  - 10mM HEPES
- **Enzyme buffer** (for DNAse and Collagenase D digestions; Stable for up to 1 month in the fridge):
  - PBS *with* Ca2+ and Mg2+ ***OR***
  - HBSS 1X *with* Ca2+/Mg2+
  - 2% CS (v/v);
  - 10mM HEPES
- **RBC Lysis buffer**: Prepare a 1X solution in $ddH_20$ from lysis buffer.
- **Stopping buffer** (require 300 µl per sample):
  - 1x PBS without Ca2+ and without Mg2+ or HBSS
  - 0.1 M EDTA (37.5 g/L)
- **Antibody cocktails for Panels 1 & 2**
  - Protect antibodies and prepared cocktails from direct light.
  - Mastermix concentration, storage temperature and stability to be determined after panels 1 and 2 have been finalised and tested.

- Each sample will require 50 µl (or up to 100 µl) of diluted 1X antibody cocktail.
- Antibody cocktails should be gently but thoroughly mixed or quickly vortexed 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 8°C prior to staining cells.

- **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 per each spleen, containing final concentrations of:
  - DNAse I: 30 g
  - Collagenase D: 600 Mandl Units

  ***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 m Nylon mesh filter into a 50 mL Falcon tube ***OR***

    - directly from C-tubes pour splenocyte suspension through 30 mm 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+ (0.17-0.2 Wünsch unit/ml)
- Mince into fine pieces using small scissors, place on ice until all samples are minced.
- Add 2ml collagenase (0.17-0.2 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 down 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 ml 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 ml 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 ml 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 ml 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 ml of 1:100 Fc block and incubate on ice for 10 min. Top up to 200 ml 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.
- Centrifuge plate, discard supernatant and resuspend in 50 to 100 ml 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 ml with FACS buffer after incubation. Centrifuge, discard supernatant and resuspend in 200 ml FACS buffer.
    - When ready to read plate, centrifuge again and discard supernatant. Resuspend the pellet in 200 ml 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 ml of PBS (RT) to all samples
    - Spin at 2000 rpm for 1 minute 8°C
    - Add 100 ml/well of Zombie Near-IR Live/Dead dye (1/2000) made up in PBS incubate at room temperature for 10 mins, add 200 ml 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 of low frequencies (macrophages, DCs).

**Gating Panel 1**

| Parameters | Gating steps | | | |
|---|---|---|---|---|
| Panel A live leukocyte count | | | | |
| T cells (panel A) | number of live leukocytes | CD5+ | CD161- | |
| NKT cells (panel A) | number of live leukocytes | CD5+ | CD161+ | |
| NK cells (panel A) | number of live leukocytes | CD5- | CD161+ | |
| Others | number of live leukocytes | CD5- | CD161- | |
| CD4 T cells | number of live leukocytes | CD5+ | CD161- | CD |
| CD8 T cells | number of live leukocytes | CD5+ | CD161- | CD |
| DN T cells | number of live leukocytes | CD5+ | CD161- | CD |
| DP T cells | number of live leukocytes | CD5+ | CD161- | CD |
| CD4 NKT cells | number of live leukocytes | CD5+ | CD161+ | CD |
| CD8 NKT cells | number of live leukocytes | CD5+ | CD161+ | CD |

| | | | | |
|---|---|---|---|---|
| DN NKT cells | number of live leukocytes | CD5+ | CD161+ | CD |
| CD4 CD25+ T cells | | number of CD5+ | CD161- | CD |
| CD4 CD25- T cells | | number of CD5+ | CD161- | CD |
| CD8 CD25+ T cells | | number of CD5+ | CD161- | CD |
| CD8 CD25- T cells | | number of CD5+ | CD161- | CD |
| DN CD25+ T cells | | number of CD5+ | CD161- | CD |
| DN CD25- T cells | | number of CD5+ | CD161- | CD |
| CD4 CD25+ NKT cells | | number of CD5+ | CD161+ | CD |
| CD4 CD25- NKT cells | | number of CD5+ | CD161+ | CD |
| CD8 CD25+ NKT cells | | number of CD5+ | CD161+ | CD |
| CD8 CD25- NKT cells | | number of CD5+ | CD161+ | CD |
| DN CD25+ NKT cells | | number of CD5+ | CD161+ | CD |
| DN CD25- NKT cells | | number of CD5+ | CD161+ | CD |
| CD4 CD44+CD62L- T cells | | number of CD5+ | CD161- | CD |
| CD4 CD44+CD62L+ T cells | | number of CD5+ | CD161- | CD |
| CD4 CD44-CD62L+ T cells | | number of CD5+ | CD161- | CD |
| CD4 CD44-CD62L- T cells | | number of CD5+ | CD161- | CD |
| CD8 CD44+CD62L- T cells | | number of CD5+ | CD161- | CD |
| CD8 CD44+CD62L+ T cells | | number of CD5+ | CD161- | CD |
| CD8 CD44-CD62L+ T cells | | number of CD5+ | CD161- | CD |
| CD8 CD44-CD62L- T cells | | number of CD5+ | CD161- | CD |
| DN CD44+CD62L- T cells | | number of CD5+ | CD161- | CD |
| DN CD44+CD62L+ T cells | | number of CD5+ | CD161- | CD |
| DN CD44-CD62L+ T cells | | number of CD5+ | CD161- | CD |
| DN CD44-CD62L- T cells | | number of CD5+ | CD161- | CD |
| CD4 CD44+CD62L- NKT cells | | number of CD5+ | CD161+ | CD |
| CD4 CD44+CD62L+ NKT cells | | number of CD5+ | CD161+ | CD |
| CD4 CD44-CD62L+ NKT cells | | number of CD5+ | CD161+ | CD |
| CD8 CD44+CD62L- NKT cells | | number of CD5+ | CD161+ | CD |
| CD8 CD44+CD62L+ NKT cells | | number of CD5+ | CD161+ | CD |
| CD8 CD44-CD62L+ NKT cells | | number of CD5+ | CD161+ | CD |
| DN CD44+CD62L- NKT cells | | number of CD5+ | CD161+ | CD |
| DN CD44+CD62L+ NKT cells | | number of CD5+ | CD161+ | CD |
| DN CD44-CD62L+ NKT cells | | number of CD5+ | CD161+ | CD |

**Gating Panel B**

| Parameters | Gating steps | | | | |
|---|---|---|---|---|---|
| Panel B live leukocyte count | | | | | |
| Neutrophils | Live | CD11b+ | Ly6G+ | | |
| Monocytes | Not Granulocytes | CD11b+ | Ly6C High | | |
| Eosinophils | Not Monocytes | CD11b+ | SSC-H High | | |
| NK Cells (panel B) | Not Eosinophils | CD161+ | CD19- | CD5- | |
| NK Subsets (Q1) | Not Eosinophils | CD161+ | CD19- | CD5- | C |
| NK Subsets (Q2) | Not Eosinophils | CD161+ | CD19- | CD5- | C |
| NK Subsets (Q3) | Not Eosinophils | CD161+ | CD19- | CD5- | C |

| | | | | | |
|---|---|---|---|---|---|
| NK Subsets (Q4) | Not Eosinophils | CD161+ | CD19- | CD5- | C |
| NKT Cells (panel B) | Not Eosinophils | CD161+ | CD19- | CD5+ | |
| NKT Subsets (Q1) | Not Eosinophils | CD161+ | CD19- | CD5+ | C |
| NKT Subsets (Q3) | Not Eosinophils | CD161+ | CD19- | CD5+ | C |
| T Cells (panel B) | Not Eosinophils | CD161- | CD5+ | | |
| T Subset | Not Eosinophils | CD161- | CD5+ | Ly6C+ | |
| B Cells | Not Eosinophils | MHCII+ | CD19+ | | |
| B1B Cells | Not Eosinophils | MHCII+ | CD19+ | CD5+ | |
| B2B Cells | Not Eosinophils | MHCII+ | CD19+ | CD5- | |
| Follicular B Cells | Not Eosinophils | MHCII+ | CD19+ | CD5- | C |
| pre-B Cells | Not Eosinophils | MHCII+ | CD19+ | CD5- | C |
| MZB | Not Eosinophils | MHCII+ | CD19+ | CD5- | C |
| cDCs | Not Eosinophils | MHCII+ | CD19- | CD11c+ | |
| cDCs CD11b Type | Not Eosinophils | MHCII+ | CD19- | CD11c+ | C |
| pDCs | Not Eosinophils | Not T Cells | Ly6C+ | CD317+ | |
| RP Macrophage (F4/80+) | Not Eosinophils | MHCII+ | F4/80+ | | |
| or | | | | | |
| RP Macrophage (CD19-CD11c-) | Not Eosinophils | MHCII+ | CD19- | CD11c- | |

## Parameters and Metadata

### **Others** KMPCLA_IMM_006_001 | v1.4

simpleParameter

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

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### **DN CD44-CD62L+ T cells** KMPCLA_IMM_038_001 | v1.3

simpleParameter

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

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## Enzyme buffer catalog number KMPCLA_IMM_115_001 | v1.1

procedureMetadata

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

**Options:** 14025,

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## DN CD25- T cells KMPCLA_IMM_019_001 | v1.5

simpleParameter

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

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## FACS buffer catalog number KMPCLA_IMM_113_001 | v1.1

procedureMetadata

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

**Options:** 14175,

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## cDCs CD11b Type KMPCLA_IMM_073_001 | v1.2

simpleParameter

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

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## Percentage of live gated events in Panel A KMPCLA_IMM_002_001 | v1.7

simpleParameter

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

**Unit Measured:** %

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## CD4 CD44+CD62L+ NKT cells KMPCLA_IMM_041_001 | v1.2

simpleParameter

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

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## FCS repository reference (URL/ID) KMPCLA_IMM_095_001 | v1.0

procedureMetadata

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

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## Dead cell exclusion dye manufacturer KMPCLA_IMM_104_001 | v1.0

procedureMetadata

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

**Options:** Sigma, home brew, Biolegend, Life Technologies,

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## Monocytes KMPCLA_IMM_051_001 | v1.2

simpleParameter

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

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## Neutrophils KMPCLA_IMM_050_001 | v1.2

simpleParameter

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

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## DP T cells KMPCLA_IMM_010_001 | v1.2

simpleParameter

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

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## Balanced salt solution catalog number KMPCLA_IMM_098_001 | v1.1

procedureMetadata

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

**Options:** 041-20211, 14175-095, HBSS 1X 14170-088, L 182-10, D1408, H6136-1L, 14190-144,

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## Panel B FCS file(s) KMPCLA_IMM_108_001 | v1.0

seriesMediaParameter

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

**Increments:** Minimum 1

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## NK Subsets (Q3) KMPCLA_IMM_056_001 | v1.2

simpleParameter

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

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## CD4 CD44+CD62L- T cells KMPCLA_IMM_028_001 | v1.2

simpleParameter

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

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## Cell digestion KMPCLA_IMM_082_001 | v1.0

procedureMetadata

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

**Options:** GentleMACS, manual,

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## NKT Cells (panel B) KMPCLA_IMM_058_001 | v1.2

simpleParameter

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

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## CD8 CD25+ NKT cells KMPCLA_IMM_022_001 | v1.5

simpleParameter

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

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## Cell counting equipment model KMPCLA_IMM_088_001 | v1.0

procedureMetadata

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

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

## RP Macrophage (CD19- CD11c-) KMPCLA_IMM_076_001 | v1.1

simpleParameter

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

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## Cell digestion agent KMPCLA_IMM_083_001 | v1.0

procedureMetadata

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

**Options:** Collagenase II, Collagenase D,

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## RPMI catalog number KMPCLA_IMM_100_001 | v1.1

procedureMetadata

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

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

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## DN CD44+CD62L+ T cells KMPCLA_IMM_037_001 | v1.3

simpleParameter

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

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## CD4 CD25- NKT cells KMPCLA_IMM_021_001 | v1.4

simpleParameter

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

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## B1B Cells KMPCLA_IMM_064_001 | v1.2

simpleParameter

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

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## Cell digestion agent catalog number KMPCLA_IMM_085_001 | v1.2

procedureMetadata

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

**Options:** CLS2LS004176, 17101-015, #11088858001,

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## CD4 CD44-CD62L+ T cells KMPCLA_IMM_030_001 | v1.2

simpleParameter

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

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## NKT Subsets (Q3) KMPCLA_IMM_060_001 | v1.2

simpleParameter

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

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## Follicular B Cells (CD21/35+) KMPCLA_IMM_067_001 | v1.1

simpleParameter

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

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## DN CD44-CD62L+ NKT cells KMPCLA_IMM_048_001 | v1.3

simpleParameter

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

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## DNAse I catalog number KMPCLA_IMM_102_001 | v1.2

procedureMetadata

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

**Options:** D8764, DN25,

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## DN CD25- NKT cells KMPCLA_IMM_025_001 | v1.2

simpleParameter

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

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## Enzyme buffer manufacturer KMPCLA_IMM_114_001 | v1.1

procedureMetadata

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

**Options:** Life Technologies,

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## Collection buffer manufacturer KMPCLA_IMM_110_001 | v1.2

procedureMetadata

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

**Options:** Life Technologies,

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## CD8 T cells KMPCLA_IMM_008_001 | v1.4

simpleParameter

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

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## DN CD44+CD62L- T cells KMPCLA_IMM_036_001 | v1.3

simpleParameter

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

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## Dead cell exclusion dye KMPCLA_IMM_103_001 | v1.0

procedureMetadata

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

**Options:** Sytox Green, Propidium Iodide, DAPI, Sytox Blue, Zombie NIR,

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## Cell lysis buffer manufacturer KMPCLA_IMM_090_001 | v1.2

procedureMetadata

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

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

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## Dead cell exclusion dye catalog number KMPCLA_IMM_105_001 | v1.1

procedureMetadata

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

**Options:** S11348, S34857, home brew, D9542, S-34860, R37606, 423106,

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## Equipment name KMPCLA_IMM_077_001 | v1.0

procedureMetadata

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

**Options:** Flow cytometer, FACS, LSR II, Fortessa_1,

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## NKT cells (panel A) KMPCLA_IMM_004_001 | v1.5

simpleParameter

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

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## Cell digestion agent manufacturer KMPCLA_IMM_084_001 | v1.1

procedureMetadata

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

**Options:** Gibco, Worthington, Roche,

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## CD4 CD25+ T cells KMPCLA_IMM_014_001 | v1.4

simpleParameter

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

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## RP Macrophage (F4/80+) KMPCLA_IMM_075_001 | v1.1

simpleParameter

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

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## MZB KMPCLA_IMM_070_001 | v1.2

simpleParameter

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

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## Date and time of sample preparation KMPCLA_IMM_093_001 | v1.0

procedureMetadata

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

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## Cell lysis buffer catalog number KMPCLA_IMM_091_001 | v1.2

procedureMetadata

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

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

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## T Subset KMPCLA_IMM_062_001 | v1.2

simpleParameter

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

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## Balanced salt solution manufacturer KMPCLA_IMM_097_001 | v1.0

procedureMetadata

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

**Options:** Biochrom, Life Technologies, Wisent, Wako, Gibco, Sigma,

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## CD8 CD44+CD62L+ NKT cells KMPCLA_IMM_044_001 | v1.3

simpleParameter

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

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## Sample storage temperature until analysis (in Celsius) KMPCLA_IMM_094_001 | v1.0

procedureMetadata

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

**Unit Measured:** C

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## CD4 CD44-CD62L+ NKT cells KMPCLA_IMM_042_001 | v1.3

simpleParameter

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

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## CS&T Bead lot KMPCLA_IMM_080_001 | v1.0

procedureMetadata

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

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## Cell counting equipment name KMPCLA_IMM_089_001 | v1.0

procedureMetadata

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

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## CD8 CD44-CD62L+ T cells KMPCLA_IMM_034_001 | v1.2

simpleParameter

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

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## T Cells (panel B) KMPCLA_IMM_061_001 | v1.2

simpleParameter

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

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## NK Cells (panel B) KMPCLA_IMM_053_001 | v1.3

simpleParameter

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

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## B2B Cells KMPCLA_IMM_065_001 | v1.2

simpleParameter

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

## CD4 CD44-CD62L- T cells KMPCLA_IMM_031_001 | v1.2

simpleParameter

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

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## Equipment manufacturer KMPCLA_IMM_078_001 | v1.0

procedureMetadata

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

**Options:** BD Biosciences, Beckman Coulter,

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## CD8 CD44-CD62L+ NKT cells KMPCLA_IMM_045_001 | v1.3

simpleParameter

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

---

## Panel A FCS file(s) KMPCLA_IMM_107_001 | v1.0

seriesMediaParameter

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

**Increments:** Minimum 1

---

## Transitional B Cells (CD21/35 low) KMPCLA_IMM_069_001 | v1.2

simpleParameter

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

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## NKT Subsets (Q1) KMPCLA_IMM_059_001 | v1.2

simpleParameter

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

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## NK Subsets (Q4) KMPCLA_IMM_057_001 | v1.2

simpleParameter

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

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## CD8 NKT cells KMPCLA_IMM_012_001 | v1.5

simpleParameter

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

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## DN CD25+ T cells KMPCLA_IMM_018_001 | v1.5

simpleParameter

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

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## T cells (panel A) KMPCLA_IMM_003_001 | v1.5

simpleParameter

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

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## Date and time of sacrifice KMPCLA_IMM_092_001 | v1.0

procedureMetadata

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

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## CD8 CD44+CD62L- T cells KMPCLA_IMM_032_001 | v1.2

simpleParameter

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

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## Total number of acquired events in Panel A KMPCLA_IMM_026_001 | v1.4

simpleParameter

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

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## Total number of acquired events in Panel B KMPCLA_IMM_027_001 | v1.2

simpleParameter

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

---

## RPMI manufacturer KMPCLA_IMM_099_001 | v1.0

procedureMetadata

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

**Options:** Sigma, Wako, Gibco, Jax, none used, Life Technologies,

---

## Spleen weight KMPCLA_IMM_001_001 | v1.0

simpleParameter

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

**Unit Measured:** g

## Equipment model KMPCLA_IMM_079_001 | v1.0

procedureMetadata

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

**Options:** CANTO-II, FACSAria III, Gallios, BD LSRFortessa Cell Analyzer, H47100123, BD LSR-II,

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## DN CD44+CD62L+ NKT cells KMPCLA_IMM_047_001 | v1.2

simpleParameter

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

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## MZB (CD21/35 high) KMPCLA_IMM_071_001 | v1.1

simpleParameter

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

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## NK cells (panel A) KMPCLA_IMM_005_001 | v1.5

simpleParameter

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

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## CD8 CD44+CD62L+ T cells KMPCLA_IMM_033_001 | v1.2

simpleParameter

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

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## CD4 T cells KMPCLA_IMM_007_001 | v1.4

simpleParameter

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

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## Automated analysis KMPCLA_IMM_109_001 | v1.0

procedureMetadata

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

**Options:** Yes, No,

---

## Cell counting KMPCLA_IMM_086_001 | v1.1

procedureMetadata

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

**Options:** post-lysis, pre-lysis,

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## CD4 CD25- T cells KMPCLA_IMM_015_001 | v1.4

simpleParameter

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

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## CD8 CD25- T cells KMPCLA_IMM_017_001 | v1.4

simpleParameter

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

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## DNAse I manufacturer KMPCLA_IMM_101_001 | v1.1

procedureMetadata

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

**Options:** Sigma,

---

## Eosinophils KMPCLA_IMM_052_001 | v1.2

simpleParameter

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

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## DN CD44-CD62L- T cells KMPCLA_IMM_039_001 | v1.3

simpleParameter

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

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## CD4 CD44+CD62L+ T cells KMPCLA_IMM_029_001 | v1.2

simpleParameter

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

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## CD8 CD25+ T cells KMPCLA_IMM_016_001 | v1.4

simpleParameter

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

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## CD4 CD25+ NKT cells KMPCLA_IMM_020_001 | v1.4

simpleParameter

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

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## CD8 CD44+CD62L- NKT cells KMPCLA_IMM_043_001 | v1.3

simpleParameter

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

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## Cell counting equipment manufacturer KMPCLA_IMM_087_001 | v1.0

procedureMetadata

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

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

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## Follicular B Cells KMPCLA_IMM_066_001 | v1.2

simpleParameter

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

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## Percentage of live gated events in Panel B KMPCLA_IMM_049_001 | v1.5

simpleParameter

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

**Unit Measured:** %

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## CD8 CD25- NKT cells KMPCLA_IMM_023_001 | v1.5

simpleParameter

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

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## Balanced salt solution type KMPCLA_IMM_096_001 | v1.0

procedureMetadata

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

**Options:** HBSS, PBS,

---

## DN NKT cells KMPCLA_IMM_013_001 | v1.4

simpleParameter

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

---

## FACS buffer manufacturer KMPCLA_IMM_112_001 | v1.1

procedureMetadata

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

**Options:** Life Technologies,

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## CD8 CD44-CD62L- T cells KMPCLA_IMM_035_001 | v1.2

simpleParameter

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

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## B Cells KMPCLA_IMM_063_001 | v1.2

simpleParameter

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

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## NK Subsets (Q2) KMPCLA_IMM_055_001 | v1.2

simpleParameter

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

## DN CD44+CD62L- NKT cells KMPCLA_IMM_046_001 | v1.2

simpleParameter

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

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## CD4 CD44+CD62L- NKT cells KMPCLA_IMM_040_001 | v1.2

simpleParameter

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

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## Cell digestion temperature (in Celsius) KMPCLA_IMM_106_001 | v1.1

procedureMetadata

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

**Options:** 37, RT,

---

## cDCs KMPCLA_IMM_072_001 | v1.2

simpleParameter

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

## Transitional B Cells KMPCLA_IMM_068_001 | v1.3

simpleParameter

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

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## pDCs KMPCLA_IMM_074_001 | v1.2

simpleParameter

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

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## Anesthesia KMPCLA_IMM_081_001 | v1.0

procedureMetadata

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

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

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## DN CD25+ NKT cells KMPCLA_IMM_024_001 | v1.4

simpleParameter

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

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## DN T cells KMPCLA_IMM_009_001 | v1.5

simpleParameter

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

---

## CD4 NKT cells KMPCLA_IMM_011_001 | v1.4

simpleParameter

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

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## NK Subsets (Q1) KMPCLA_IMM_054_001 | v1.3

simpleParameter

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

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## Collection buffer catalog number number KMPCLA_IMM_111_001 | v1.2

procedureMetadata

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

**Options:** 24020,