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Intestinal Lamina Propria and Spleen Immune Cell Isolation

rabdelha [1]

[1]California Institute of Technology

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rabdelha

This protocol details isolation of immune cells from intestinal lamina propria/spleen and flow cytometry.

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Immune Cells Isolation, Intestinal Lamina Propria/Spleen , Flow Cytometry

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Isolation of Immune Cells from Intestinal Lamina Propria/Spleen

1 Dissect the small and large intestines for isolation of intestinal lamina propria cells, place the small and large intestines immediately **On ice** cold PBS.

2 

Open the intestines longitudinally after removing the mesenteric fat and Peyer's patches (small intestine), wash out the luminal contents with cold PBS.

3  

35m

Wash the tissue pieces for **00:10:00** in **1 millimolar (mM)** dithiothreitol (DTT)/PBS at **Room temperature** on a rocker to remove mucus, followed by a wash for **00:25:00** in **10 millimolar (mM)** EDTA/**30 millimolar (mM)** HEPES/PBS at **37 °C** on a platform shaker (**180 rpm**) to remove epithelium.

4 

1h 32m

After a **00:02:00** wash in complete RPMI, digest the tissue in a 6-well plate for **01:30:00** in complete RPMI with **150 U/ml** (small intestine) or **300 U/ml** (large intestine) collagenase VIII (Sigma) and **150 µg/µL** DNase (Sigma) in a cell culture incubator (5% CO_2).

5 

20m

Pass the tissue digests through a **100 µm** cell strainer and separate them by centrifugation (**1200 x g** for **00:20:00**) using a 40/80% percoll gradient.

6 Collect the immune cells at the 40/80% interface.

7 

8m

For the spleen, pass the tissue through a **100 µm** cell strainer and incubate in red cell lysis

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buffer (Sigma) for **00:08:00** at **Room temperature** .

8

Wash both spleen and intestine immune cells with 0.5% BSA/PBS before staining and fixation (eBioscience Foxp3 / Transcription Factor Staining Buffer Set).

Flow Cytometry

9 Use , CD16/32 antibody (eBioscience) for flow cytometry staining to block the non-specific binding to Fc receptors before surface staining.

10 Isolate the immune cells from intestinal lamina propria and stain with antibodies against the following markers:

A	B
Marker	**Stain**
CD103	PerCP-efluor710
CD11b	SuperBright645
CD11c	FITC
CD19	FITC
CD3e	PE
CD4	APC
CD45.2	BV421
CD64	APC-Cy7
CD8a	APC-e780
CSF1R	PE
Ly6C	APC
MHCII I-A/I-E	PE or PerCP-efluor710
TCRβ	PerCP-Cy5.5

11 For some panels, a lineage marker mix (Lin) contained TCRβ, B220, Ly6G and Siglec-F (PE-Cy7).

12 Discriminate the live and dead cells by Live/Dead Fixable Aqua Dead Cell Stain Kit (Invitrogen).

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