

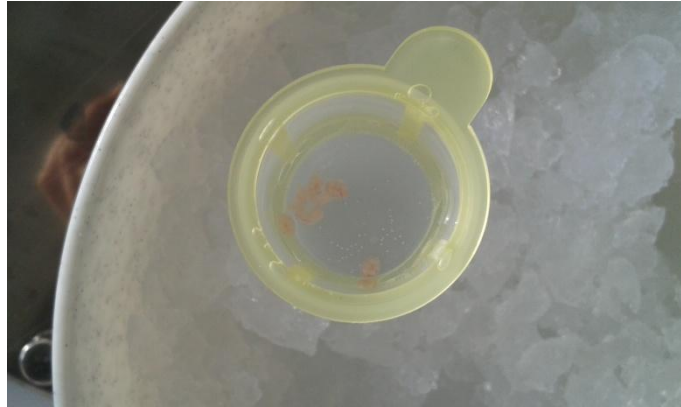
Payer's Patches Lymphocyte Isolation

Method:

1. Remove the small intestine and put it in a petridish on ice containing 1xDPBS
2. Remove Peyer's patches using a dissection scissor.



3. All patches collect into the 50 mL centrifuge tube contain ice cold DPBS.



4. Mash the Payer's patches through the cell strainer until just the connective tissue remain on the strainer.



5. Centrifuge the cell suspension at $250 \times g$, at 4°C for 5 minutes. Remove the supernatant and resuspended the cells 1 mL cold DPBS than fill up the tube up to 50 mL. (Washing step). Centrifuge again at $250 \times g$, at 4°C for 5 minutes.

6. Discard the supernatant and resuspended the cells in culture medium (IMDM or RPMI).

7. Cells can now be used for FACS analysis, PMA/Ionomycin or peptide stimulation, etc...

Typical smear picture:



FACS analysis:

Tube: Lymph

Population	#Events	%Parent	%Total
All Events	20,000	###	100.0
singlets	16,288	81.4	81.4
Live	9,724	59.7	48.6
CD45	9,546	98.2	47.7
Lymphocytes	8,986	94.1	44.9
CD3+	7,249	80.7	36.2
CD8a+	5,319	73.4	26.6
CD8a+ CD4+	859	11.8	4.3
CD8a- CD4-	927	12.8	4.6
CD4+	144	2.0	0.7

