**Anti-iNKT MicroBeads Isolation protocol**

*Ajit N Shah, Rachel Hatano*

1. Determine cell number.

2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.

3. Resuspend cell pellet in 400 μL of buffer per 10⁸ total cells. Add 100 μL of Anti-iNKT MicroBeads per 10⁸ total cells.

4. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
5 Wash cells by adding 1–2 mL of buffer per 10^8 cells and centrifuge at 300xg for 10 minutes. Aspirate supernatant completely.

6 Resuspend up to 10^8 cells in 500 μL of buffer.

7 Place column in the magnetic field of a suitable MACS Separator.

8 Prepare column by rinsing with the appropriate amount of buffer: LS: 3 mL

9 Apply cell suspension onto the column. Collect flow-through containing unlabeled cells.

10 Wash column with the appropriate amount of buffer. Collect unlabeled cells that pass through and combine with the effluent. LS: 3×3 mL.

11 Remove column from the separator and place it on a suitable collection tube.

12 Pipette the appropriate amount of buffer onto the column.

13 Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column. LS: 5 mL

14 To increase the purity of iNKT cells, the eluted fraction is enriched over a second MS or LS Column.

Citation: Ajit N Shah, Rachel Hatano (12/10/2019). Anti-iNKT MicroBeads Isolation protocol. https://dx.doi.org/10.17504/protocols.io.bacuiaww

This is an open access protocol distributed under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.