Anti-iNKT MicroBeads Isolation protocol

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1 Works for me

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ABSTRACT

isolation of 6b11 positive cells

MATERIALS

NAME	CATALOG #	VENDOR
Anti-iNKT MicroBeads human	130-094-842	Miltenyi Biotec

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 10^8 total cells. Add 100 µL of Anti-iNKT MicroBeads per 10^8 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 300×g 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.