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Anti-iNKT MicroBeads Isolation protocol

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ABSTRACT

isolation of 6b11 positive cells

MATERIALS

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Anti-iNKT MicroBeads human **Miltenyi Biotec Catalog #130-094-842**

OPEN ACCESS

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Protocol status: Working
We use this protocol and it's working

Created: Dec 10, 2019

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PROTOCOL integer ID:
30836

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 10⁸ cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
- 6 Resuspend up to 10⁸ 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.