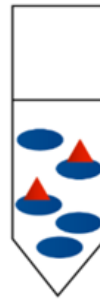


abcam

Intracellular flow cytometry protocol summary

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Ice-cold samples containing 1×10^5 – 1×10^6 cells per 100 μ L.



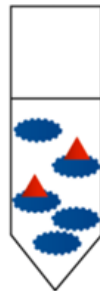
Legend:

- Cells
- Antigen
- Fluorochrome conjugated antibody

Add 100 μ L fixative to each tube. Incubate for 15 min at 4°C.

Wash four times with PBS 0.2% Tween 20, centrifuge at 400G for 5 min.

Permeabilize: add 100 μ L permeabilizing agent (0.2% saponin or 0.2% digitonin) to each tube. Incubate for 15 min at 4°C.



Wash four times with PBS 0.2% Tween 20, centrifuge at 400G for 5 min.

Block with 5% serum or BSA for 15–30 min.

Wash four times with PBS 0.2% Tween 20, centrifuge at 400 x g for 5 min.

Incubate with 0.1–10 μ L/mL fluorochrome-conjugated primary antibody for 15–45 min at 4°C in the dark.



Wash four times with PBS 0.2% Tween 20, centrifuge at 400G for 5 min.

Resuspend in ice-cold PBS, 10 FCS and 1% sodium azide to a final volume of 0.5–1 mL. Store at 4°C - until analysis (within 24 h).

Run and analyze on flow cytometer.