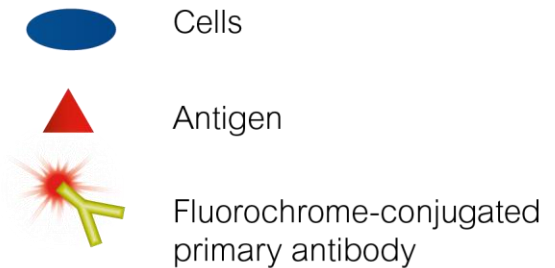
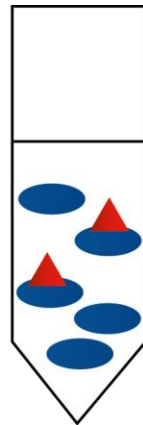


# Flow cytometry membrane staining protocol summary

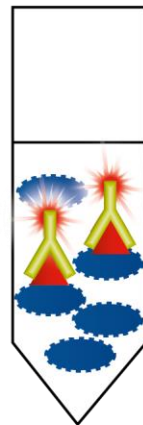
Ice cold sample containing  $1 \times 10^5$ – $1 \times 10^6$  cells per 100  $\mu$ L.



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



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

Fix (optional): Add 100  $\mu$ L fixative (e.g. 4% PFA) to each tube and incubate for 15 min at 4°C.

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

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

Run and analyze on flow cytometer.