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NBP2-44248 Protocol

Flow Cytometry protocol for CD133 Antibody (NBP2-44248)

CD133 Antibody: https://www.novusbio.com/products/cd133-antibody_nbp2-44248
General Intracellular Cytoplasmic Target Flow Protocol
1. Grow cells to 50-75% confluency. Flow cytometry requires between 2 x 105 and 1 x 106 cells for optimal performance.
2. If cells are adherent, harvest gently with 1X PBS and scraping. Avoid using trypsin as this can disrupt certain epitopes of interest. If enzymatic harvest is required, use Accutase or Collagenase for a less damaging option.
3. Reserve 100 uL for counting, then transfer cells into a 50mL conical tube and centrifuge for 8 minutes at 400 RCF (~1200 RPM)
4. Re-suspend cells to a concentration of 1 x 106 cells/mL in Flow Buffer (1X PBS + 0.5% BSA)
5. Aliquot out 1 mL samples in accordance with your experiment.
6. Permeabilize cells by adding 100uL of a permeabiliation buffer to every 1 x 106 cells present in the sample. Mix well and incubate at room temperature for 15 minutes.
a. For cytoplasmic targets, use a gentle permeabilization solution such as 1X PBS + 0.5% Saponin or 0.5% Tween 20
b. To maintain the permeabilized state throughout your experiment, use Flow Buffer + 0.1% of the permeabilization chemical (i.e. 0.1% Tween 20 or 0.1% Saponin)
7. Following the 15 minute incubation, add 2 mL of the Flow Buffer + 0.1% permeabilizer solution to each sample.
8. Centrifuge for 5 minutes at 400 RCF (~1200 RPM)
9. Discard supernatant and re-suspend in 1 mL of Flow Buffer + 0.1% permeabilizer
10. Stain each sample at 1 uL/ 1 x 106 cells of primary antibody or 1-3 uL/ 1 x 106 cells for directly conjugated antibodies. Mix well and incubate on ice for 30 min- 1 hour. Gently mix samples every 10-15 minutes
11. Following the primary/conjugate incubation, add 2 mL/sample of Flow Buffer+0.1% permeabilizer and centrifuge for 5 min at 400 RCF (~1200RPM)
12. Remove supernatant and re-suspend each sample in 2 mL Flow Buffer+0.1% permeabilizer, repeat wash for 5 minutes at 400 RCF (~1200 RPM)
13. The next step differs based on experimental design:
a. If using a fluorescent secondary, re-suspend the pellet in 1 mL Flow Buffer+0.1% permeabilizer and add 1 uL secondary to each sample. Mix and incubate on ice for 20 minutes.
b. If using a directly conjugated antibody, after the 2nd wash, re-suspend cell pellet to a final volume of 500 uL per sample and proceed with flow analysis.
14. In the primary-secondary experimental design, following the 20 minute secondary incubation on ice, add 1 mL Flow Buffer+0.1% permeabilizer to each sample and centrifuge for 5 minutes at 400 RCF (~1200 RPM)
15. Remove supernatant and add 2 mL Flow Buffer+0.1% permeabilizer per sample and repeat wash for 5 minutes at 400 RCF (~1200 RPM)
16. Re-suspend cell pellet to a final volume of 500 uL in Flow Buffer+0.1% permeabilizer. Proceed with flow analysis.