Cell Depletion
with Akadeum Microbubbles

1. Prepare your cells

1.1 Homogenize tissue and lyse RBCs as needed.

1.2 Centrifuge cell suspension (5 min, RT, 400 x g), aspirate supernatant, and wash once with Separation Buffer.

1.3 Centrifuge samples (5 min, RT, 400 x g) and aspirate supernatant.

1.4 Resuspend cells in Separation Buffer and transfer to 5 mL Eppendorf tubes.

Recommended supplies:

1. Cold (2-8°C) Separation Buffer (Ca^{2+} and Mg^{2+} free PBS containing 2 mM EDTA and 0.5% biotin free BSA)
2. Low retention 1 mL pipet tips (VWR Part #89174-530)
3. Centrifuge with swinging bucket rotor

2. Label cells

2.1 Add biotin-antibody cocktail. Mix briefly.

2.2 Incubate sample for 20 min at 4°C.

2.3 Add Separation Buffer and centrifuge (5 min, RT, 400 x g). Aspirate supernatant.

2.4 Resuspend cells in Separation Buffer.

3. Bind microbubbles

3.1 Prepare microbubbles by resuspending in solution (microbubble mixture should be a homogenous white solution, *i.e.* look like milk). Vigorously mix or pipet. Immediately proceed to next step.

3.2 Add microbubbles to first sample.

3.3 Set the pipet volume to ~70% of the total sample volume (cell suspension + microbubbles) and mix microbubbles with gentle trituration for 30 pipet strokes using low retention 1000 µL pipet tip.

Note: This pipet setting ensures adequate mixing of microbubbles and cells for binding.

3.4 Immediately add Separation Buffer.

Note: This faciliatates separation of microbubbles from the cell pellet.

3.5 Repeat steps 3.1–3.4 for remaining samples making sure to **resuspend microbubbles before each sample.**

4. Separate cells

4.1 Centrifuge samples (5 min, RT, 400 x g).

Note: Use of a swinging bucket rotor for this step facilitates microbubble aspiration.

4.2 Aspirate off white microbubble layer and supernatant. Take care not to aspirate cell pellet.

4.3 Resuspend cells in Separation Buffer. Transfer cells to a new tube if desired due to residual microbubbles stuck to tube.

4.4 Continue to flow cytometry or other downstream processing or handling.

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