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INNOVATIONS FOR ON-SITE SAMPLE PREP
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Protocol for Thawing and Lysing Erythrocytes in Whole Blood Samples Banked with Bulk-supplied Smart Tube Proteomic Stabilizer PROT1
Protocol Number: STTLTF-130528B

Required buffers:

1X Thaw-Lyse (dilute 1000X concentrate with distilled water to make 1X working concentration).
Buffer should be at room temperature

Staining Buffer (ie. Phosphate Buffered Saline + 0.5% Bovine Serum Albumin)
Buffer should be at 4°C

Other buffers that may be required:

1X Lyse Buffer 2 (dilute 5X concentrate with distilled water to make 1X working concentration).
Buffer should be at room temperature.

Protocol:

1. If samples were not frozen because analysis will take place same-day, proceed with Step 2. Thaw frozen samples in a cold water bath (10°C to 15°C) with agitation of the water (ie. a rotating magnetic stir bar or use of an orbital shaker) for approximately 20 minutes until the samples are fully thawed. Alternatively, thaw tubes in an open rack in 4°C refrigerator for approximately 30 minutes until the samples are fully thawed; do not use a Styrofoam rack to hold samples as it is important for samples to transition quickly from -80°C to fully thawed state. Once the samples are thawed promptly move to the next step.

2. Add 1X Thaw-Lyse Buffer at a ratio of 4:1 to the total volume of the stabilized sample (ie. if the original sample was 1ml of whole blood, 1.4ml of stabilizer was added to give a total volume of 2.4ml after stabilization; to this add at least 9.6ml of 1X Thaw-Lyse Buffer). Mix thoroughly and incubate the samples at room temperature for 10 minutes.

3. Centrifuge at 600 x g for 5 minutes at room temperature to pellet the leukocytes. Discard the supernatant.

4. Resuspend the pellet in 25ml of 1X Thaw-Lyse Buffer and incubate at room temperature for 10 minutes. Centrifuge the conical tubes at 600 x g for 5 minutes at room temperature. Discard supernatant. If the pellet is relatively white, erythrocyte lysis is complete.

5. Erythrocyte lysis complete, the sample should be resuspended in staining media and transferred to a FACS tube or plate and washed with staining media. Centrifuge the sample as before at 600 x g for 5 minutes. The sample can now be stained as if it were leukocytes that had been fixed with paraformaldehyde using standard staining protocols for fixed cells. Note that to carry out intracellular staining, the cells must be permeabilized with alcohol (ie. 90% methanol) or detergent (ie. 0.2% saponin) as the Smart Tube system, like conventional fixation approaches, yields cells whose membranes are somewhat permeable to small molecule dyes such as propidium iodide, but are otherwise intact and do not provide suitable antibody access to the inside of the cell without further permeablization by alcohol or detergent.

Notes

It is recommended that the conditions used to thaw the sample (temperature of the water bath, agitation, and duration) be standardized across all samples in a study.

Complete erythrocyte lysis is typically achieved after Step #4. Longer Stabilization Times at room temperature or prolonged incubation of the samples at 4°C (or higher temperatures) after they have thawed may make erythrocyte lysis more difficult.

In the event that erythrocyte lysis is not complete after Step #4, follow these steps:

A1. Resuspend the pellet in >15ml of 1X Lyse Buffer 2, incubate for 10 minutes at room temp, pellet as before and discard supernatant. If the pellet is relatively white indicating erythrocyte lysis is complete, proceed with Step #5 above. If not, continue with Step A2 below.

A2. Resuspend the pellet in >15ml of 1X Thaw-Lyse Buffer, incubate for 10 minutes at room temp, pellet as before and discard supernatant. Pellet should be relatively white indicating erythrocyte lysis is complete. Proceed with Step #5 above.