SMART TUBE Inc.
INNOVATIONS FOR ON-SITE SAMPLE PREP
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Protocol for Thawing Samples Frozen In Smart Tubes
Protocol Number: STTLNB-131103R170913-RevA

Required Items:

Processing Insert, one for each Smart Tube

1X Thaw-Lyse (dilute 1000X concentrate with ddH2O to make 1X working concentration). This buffer should be at room temperature

Staining Buffer (ie. Phosphate Buffered Saline + 0.5% BSA)

Other buffers that may be required:

1X Lyse Buffer 2 (dilute 5X concentrate with ddH2O to make 1X working concentration). This buffer should be at room temperature.

Protocol:

1. Thaw the Smart Tubes in a cold water bath (10°C to 12°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 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. A sample is not sufficiently thawed if, when shaken vigorously, there is no sound of the contents moving inside the Smart Tube. Once the samples are thawed it is important to promptly move to the next step. Note that if samples were not frozen because analysis will take place same-day, proceed with Step 2.

2. Once thawed, uncap the Smart Tube. Add the Processing Insert to the Smart Tube with a slight twisting motion to ensure the Processing Insert is firmly seated in the Smart Tube. Add ~1ml of 1X Thaw-Lyse Buffer to the Smart Tube and decant contents through a cell strainer (60 to 100 micron mesh) into a 50 ml conical tube containing 25 ml of 1X Thaw-Lyse Buffer. To get remnants of the sample from a Smart Tube, add a couple more milliliters of 1X Thaw-Lyse Buffer to the Smart Tube, vortex, and decant contents through the cell strainer. Note that there may still be red coloration to the material in the Smart Tube, but the vast majority of the leukocytes will have been decanted so additional washes of the Smart Tube are typically unnecessary. Pass an additional 5ml of 1X Thaw-Lyse Buffer through the cell strainer to ensure most of the cells in the cell strainer pass through into the 50 ml conical. Discard the cell strainer and now empty Smart Tubes and incubate the sample in the 50 ml conical at room temperature for 10 minutes.

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

4. Resuspend the pellet in 25 ml of 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 and discard supernatant. 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 permeabilization 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.

If the sample frozen in the Smart Tube is not whole blood, but rather PBMC or a cell line in suspension then carry out Steps 1 and 2 above substituting phosphate buffered saline for Thaw-Lyse Buffer and omitting the 10 minute incubation time in Step 2. Carry out Step 3 and then wash twice with Staining Buffer, pelleting the cells each time as in Step 3. Skip steps 4 and 5. The sample should now be ready to stain and/or permeabilize and stain.

If the Stabilization Reaction Time was 10 minutes or less, the samples were not left in the machine for very long after the Base Station completes the protocol, and were thawed consistent with Step #1 above, then complete erythrocyte lysis is typically achieved after Step #4. Longer Stabilization Times, leaving the samples in the Base Station for an extended time after completion of the protocol 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 20 ml 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 20ml 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.