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## Alternate Dry Ice Fixing Procedure for MicroFlow® Kits

### Materials Required:

- Dry ice (preferably small pellets).
- Styrofoam® box (with thin top and holes made to fit 15 ml polypropylene centrifuge tubes). Prepare the Styrofoam box ahead of time and save for future use. See instructions and photo below.
- 15 ml polypropylene centrifuge tubes pre-aliquoted with 2 ml methanol (Fixative). These must be pre-chilled at  $-75\text{ }^{\circ}\text{C}$  to  $-85\text{ }^{\circ}\text{C}$  freezer for at least 1 day before performing this procedure.

This alternate fixing procedure enables you to fix samples on dry ice with some precautions. It is useful if a chest freezer is not available and an upright freezer is being used. A key factor is to keep carbon dioxide vapors, caused by sublimating dry ice, from carbonating the Fixative (either before or after cells are introduced), as this can lead to excessive cellular aggregation.

### **Prepare Styrofoam® Box**

Use a Styrofoam® box with a lid that is not too thick. Punch holes in the top of the lid that are JUST big enough to fit the 15 ml polypropylene centrifuge tubes containing Fixative. Make holes on either side of the box to allow the dry ice vapors (which are heavier than air) to fall away from the container and away from the tubes holding Fixative. When the tubes are placed in the lid, the bottom 2 inches to 3 inches of each tube should be surrounded by the dry ice to maintain the Fixative at the proper temperature.

### **Fix Samples**

When ready for use, move the box close to the freezer housing the pre-aliquoted Fixative tubes. COMPLETELY fill the box with small dry ice pellets. Remove the appropriate number of tubes (this depends on the number of holes made in the lid) from the freezer, and place in the holes made in the top of the box.

Just before adding cells, loosen all caps. Fix the cells as described in the manual, except that rather than removing a tube from the freezer, lift it out of the box top. After fixation, replace and tighten the cap, and vortex or tap very vigorously as described in the manual. Return the tube to the dry ice box and continue with the other samples.

When each of the tubes in the box has had sample added, double check that all caps are VERY tightly secured and move all tubes back to the  $-75\text{ }^{\circ}\text{C}$  to  $-85\text{ }^{\circ}\text{C}$  freezer (preferably to another shelf if using an upright freezer, where the door has not been opened too many times). Continue with another batch of tubes until all samples are fixed.

Please note that this basic procedure can also be used when washing samples out of Fixative.

