Thawing cells:

1. Label flask or dish w/ "cell line name, THAW, date, and count (if necessary)".

2. Put 5ml complete media in 15ml CT.

3. Put frozen vial on empty cane and thaw in waterbath making sure not to immerse vial threads below water line.

4. Immediately (using plugged pasteur pipet) take cells from vial and transfer to 15 ml CT with media. Resuspend.

5. Spin down at medium speed for 2-3 minutes.

6. While spinning, add media to flask or dish (appropriate amount less 5ml).

7. Being careful not to aspirate cell pellet, aspirate media from 15ml CT.

8. Add 5ml fresh complete media to 15 ml CT and resuspend cell pellet. (At this point some cells may be added to isoton vial to count.)

9. Add to prepared dish or flask. Place in incubator.

10. Count cells if needed

11. Feed thaw next day and/or split as usual when ready. Note: use higher densities (lower split ratios) for first couple of passages because cells may be growing slowly. Increase split ratio when all densities are confluent. Try to create optimum confluency w/middle density.