Thawing primary leukemia cells

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This protocol is used to thaw primary cells. Key points are the addition of DNAse, which helps to preserve viability.

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- DNase- I (thawed, sitting on ice stock concentration is 1mg/mL in PBS (Sigma #D4513, 1 vial contains 10 mg that are 80% pure, i.e. reconstitute vial in 8 ml PBS and aliquot). Use each aliquot only once.
- IMDM medium + 2% FBS (cold)
- 15 ml conical tube(s)

1 Transfer the contents to a 15ml conical tube.

2 Add DNase. 1/10 of the volume of liquid in the tube (100µl/1ml of sample). Do not pipette. Gently shake the sample to mix in the DNase.

3 Incubate sample in 37˚C water bath for 90 seconds.

4 Add media. 10ml, Drop for drop or very slowly while again gently mixing the sample.

5 Spin. @1600rpm for 6-7minutes.

6 Aspirate media.

7 Add DNase, 70µl/ 1ml of sample.

8 Resuspend cells in DNase by flicking the pellet.

9 Add media, 10ml, again very slowly, then resuspend cells after adding about half of the media by pipetting very gently up and down (or inverting the tube once all the media is added)

10 Spin @ 1600rpm for 6-7 minutes.

11 Aspirate media.

12 Add DNase. 50µl per 1ml of sample.

13 Resuspend in desired media.

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14 Count cells.

15 Note: • The use of DNase is especially important if you use PBS. It will prevent a lot of clumping. Adding the medium very slowly and continous resuspending of the cells will also greatly reduce clumping.

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