Thawing and Preparation of Frozen Cells

All of our patient samples are either peripheral blood or bone marrow, and are cryopreserved in media containing 20% FBS and 20% DMSO. Vials must be thawed quickly by placing them in a 37 deg water bath for a couple of minutes.
After thawing, add cells to 15ml tubes that contain the following:

-FBS
-4µm $MgSO_4$
-5u/ml Heparin
-70u/ml DNAse
It's easiest to use the same final volume , so you can calculate the above concentrations once, and then use the same amount each time.
After adding the cells to the tubes, wash out the cryovials with media to ensure maximum yield of cells.
Allow to sit at room temp for a few minutes, then spin, decant, and resuspend cells in some fresh media.

Next, count the cells on the Coulter counter, and perform a viability assay:

Viability assays:
There are 2 easy methods-

(a) Place 20µl of cells on a slide, add 20µl Trypan Blue, and view under the microscope. Viable cells should appear round and bright, almost opaque. Dead cells will be dark blue. Estimate cell viability by comparing living and dead cells. If viability is less than 40% the sample is inevaluable.

(b) Add 10µl Propidium Iodide to a 0.5 ml cell suspension and run tube on the FACScan. This is a much more accurate method than above.

Cells are now ready to be assayed.