Cyropreservation

Cell Counting using Vi-Cell Analyzer

- Using inner wall of 50 ml conical tube, mix splenic cell suspension by drawing/expelling volume by pipetor. Take caution not to create bubbles.

- For a 30:1 dilution, draw 20 μl of splenic cell suspension and transfer to Vi-Cell tube, then add 580 μl of 1x PBS. If working with fewer cells use less suspension, but load a final volume of 600 μl. However, a 30:1 dilution is preferable.

- Centrifuge remaining 50ml conical tube volume @ '7.5' speed for 5 minutes

- Log onto Vi-Cell Analyzer using "sabanroom" for user id and "chlar413" for password. Enter sample info into fields. For leukemic cells use "small leukemia" setting, while "default" setting should be used for normal splenic suspensions.

- Print out Vi-Cell report. Calculate a total volume to resuspend between 20-40 million cells per cyropreserved vial (final volume of 1ml per vial X number of vials). If banking, resuspend 10 million cells per vial.

- Log entry/ies into –180 C Location Folder. Info should include Sample ID, Mouse #, % Viable, # of Cells/vial, Date, Number of vials, and Initials. Also record data in both a lab book and in a computer database.

- Load vials into room temp "Mr. Frosty" (current max 18 vials) and place in -80 freezer

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- Retrieve centrifuged splenic cells, and decant supernatant. Total final volume will be 1ml of Freezing Media (BioVeris 10% DMSO, Fisher Scientific Cat# IG-50-0715). Take care to avoid bubbles.

- Transfer 1 ml aliquots to cyropreservation vials

- If volume is unequal between vials, equalize first by balancing volume amongst vials;