OSUCCC LTB Laboratories Procedure Cell suspension enumeration and viability determination using the Beckman Coulter ViCELL®			Effective: 6/21/2011
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		9/25/2013	3
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		11/30/2015	3

1.0 PRINCIPLE

The Vi-CELL Series Cell Viability Analyzers provide an automatic and cost effective means to perform the Trypan blue dye exclusion method to enumerate and determine viability of a mammalian cell suspension. This data is essential to the decision making process for basic tissue culture cell passage and maintaining optimum culture conditions in bioreactors. Cell and tissue culture applications in life science research, including recombinant protein and biopharmaceutical production, require the accuracy, precision and automation available only in the Vi-CELL cell viability analyzer.

Equipment Features

% Viability
Total cell concentration
Total viable cell concentration
Mean cell size
Real time cellular images
Bioprocess monitoring
Validated reagents
Convenient reagent pack

2.0 SPECIMEN

Fresh peripheral blood, bone marrow or products of leukapheresis are collected with anticoagulation reagent (heparin, EDTA) that is specified in the sample collection protocol. Specimens should be procured as soon as they are received by the lab and preferably within 24 hours of being procured from the patient/subject.

3.0 MATERIALS AND REAGENTS

Sterile pipet tips (200µl and 1000 µl)
Micropipetters (20-200µl and 1000 µl)
ViCELL specimen cup
Beckman Coulter – Isoton Solution
70% isopropyl alcohol

4.0 EQUIPMENT

Biosafety cabinet
Beckman Coulter ViCELL automated viable cell counter

5.0 QUALITY CONTROL AND SAFETY

It is recommended that specimen collection be carried out in accordance with NCCLS document M29T2. No known test sample can offer complete assurance that human blood samples will not transmit infection. Therefore, all derivatives are potentially infectious. Always spray alcohol on the caps before opening solutions. The alcohol can be dried off using gauze. If you have been out of the hood for a while and are wearing the same pair of gloves, use a new pair of gloves. Remember not to touch the sides (inside or outside) of any bottles with your pipette – if you do, dispose of the pipette and start again.

6.0 PROCEDURE – Pre-Ficoll Cell Count – Sample Preparation

6.0.1 Log sample data into Sample Data Worksheet
6.0.2 Prepare a ViCell cup with 2ml of Isoton® II.
6.0.3 Add 40µl of mixed fresh blood or bone marrow aspirate to the prepared vial.
6.0.4 Count sample and record count on sample worksheet.
6.0.5 Cell count is given in cells/ml. To obtain total cell number, multiply cell/ml x total volume of cell suspension, e.g. count result = 1.9 x 10^7 cells/ml resuspended in 5ml PBS or 1.9x10^7cells/ml x 5 ml = 9.5x10^7 total cells.

6.1 PROCEDURE – Post-Ficoll Cell Count – Sample Preparation

6.1.1 Complete Ficoll MNC Separation according to CALGB-OSU LTB SOP[1]
6.1.2 Prepare a ViCell cup with 2 mL of Isoton® II.
6.1.3 Add 40 µL of mixed fresh blood or bone marrow aspirate to the prepared vial.
6.1.4 Count sample and record count on sample worksheet.
6.1.5 Cell count is given in cells/mL. To obtain total cell number, multiply cells/mL x total volume of cell suspension, e.g. count result = 1.9 x 10^7 cells/ml resuspended in 5ml PBS or 1.9x10^7cells/mL x 5 mL = 9.5x10^7 total cells.

7.0 VICELL COUNTING PROCEDURE

7.0.1 Turn on the computer and double click **ViCELL** icon to open program. Once the program is started up, you should hear a pump initializing the instrument.
7.0.2 Prepare sample a cup by labeling it with the sample accession number and adding 2ml Isoton counting solution.
7.0.3 Add 40µl of the diluted sample to the prepared cup.Place sample cups in carousel. The samples do not have to start in position 1
7.0.4 Click **Log in Sample** and follow on screen instructions. Type in sample accession number. Select **PBMCs** as cell type. This identifies the analysis parameters for ficolled blood and bone marrow samples. Use dilution factor of **50**. If counting more than one sample, select **Next Sample**, otherwise, select **OK**.
7.0.5 Click **Start Queue** to begin counting. Record the cell counts and viability on the appropriate worksheet. The ViCELL is self-cleaning and will dispose of the sample cups after counting. When finished, leave the ViCELL on for next use.
7.0.6 To change reagent pack and/or waste receptacle: Remove used reagent pack: Attach color-coded tubing to each corresponding reagent pack bottle. Place reagent pack inside ViCell compartment with the open window of the pack facing the door.
7.0.7 Attach waste line to waste container and place inside the ViCELL
7.0.8 Click main screen toolbar **Instrument and Replace Reagent Pack**, this allows the lines to prime and will reset the maximum number of runs.
7.0.9 If more Reagent Packs are needed outside our regular order, please contact Beckman-Coulter. **1-800-523-3713 ext. 3826**.

8.0 LIMITATIONS OF THE PROCEDURE

[1] T:\HCG\Caligiuri Lab\Procurement\Lab Manual\Protocols\CALGB-OSU\Current SOP's

8.0.1 The Vi-CELL automates the widely accepted Trypan blue cell exclusion method. Historically, cell viability determinations were performed manually using a light microscope and hemacytometer. This technique has major shortcomings due to subjective determination of cell count, as well as manual, time consuming steps. In comparison to the manual technique, the Vi-CELL automatically performs the Trypan blue with video imaging of the flow-through cell. Results are obtained in minutes.

9.0 REFERENCES

1. ViCell® Operator's Manual. Beckman Coulter Corporation, March 2003