OSUCCC LTB Laboratories Procedure Calibration of ViCell® Cell Counter using Via™Check Concentration Control (1×10^6)			Effective: 12/28/2010
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1.0 PRINCIPLE

ViaCheck™ Concentration Control particles are a suspension of ~10µm undyed microspheres that are produced at a fixed concentration of particles/milliliter. ViaCheck™ Concentration Controls are instrument standards for calibration and instrument monitoring of cell concentration (particles/ml) using Cell Viability Analyzers.

Characteristics

Bead Concentration: 1×10^6 particles/ml (0.9×10^6 - 1.1×10^6 particles/ml)
Particle Size: 9-12µm

2.0 SPECIMENS

Mononuclear cell fractions are prepared by ficoll and cells are suspended in sterile, isotonic buffer solution such as Dulbecco's PBS (Ca/Mg free). Cells are obtained from bone marrow or peripheral blood collected with an anticoagulant such as heparin or EDTA.

3.0 MEDIA AND SUPPLIES

3.1 Materials Supplied

- 20ml of ~10µm undyed microspheres in a solution of buffered salts and surfactant containing 0.08% sodium azide

3.2 Materials required but not supplied

- Precision pipets with disposable tips to deliver 20-200µl, 200-1000µl
- Isotonic Buffered Saline Diluent (optional)

4.0 EQUIPMENT

Cell Viability Analyzer or Particle Counter ex. Coulter ViCell® XR Cell Viability Analyzer or Coulter® Counter

5.0 QUALITY CONTROL AND SAFETY

All samples should be handled using universal precautions for biohazards. D-PBS supplemented with FBS can be a source of bacterial contamination. Care must be taken to insure that D-PBS/FBS solution is always handled with aseptic technique. Store product at 4-30°C. Freezing particles may result in irreversible aggregation and loss of binding activity. This particle suspension contains sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azides. Upon disposal of material, flush with a large volume of water to prevent azide accumulation. Please consult the Material Safety Data Sheet for more information. 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

For the best accuracy be sure to work carefully and quickly when sampling and pipetting ViaCheck™ particles. Allowing the particles to stand for even a short period of time could lead to inaccurate data and results.

6.0.1. Vortex and mix (inversion or tube rotator) the vial of particles to ensure a well mixed suspension.

6.0.2. Place a minimum of 0.5 - 1.0ml of the particles into an analyzer sample cup.

6.0.3. Place the sample cup in the analyzer sampling station.

6.0.4. Using the ViCell® analyzer menu, set up and save a "CELLTYPE" for Viability controls at the settings below. *Note:* These settings are guidelines to allow the user to analyze the ViaCheck™ Viability Control Particles and may have to be adjusted for each instrument.

Cell Type Viability Control
Minimum Cell Diameter 2 (µm)
Maximum Cell Diameter 50 (µm)
Minimum Circularity 0.9
Dilution Factor 1.0
Cell Brightness 70%
Cell Sharpness 75%
Viable Cell Spot Brightness 55%
Viable Cell Spot Area 1.0%
Decluster Degree Low
Aspirate Cycles 2
Trypan Blue Mixes 3

5. Analyze the sample according to the analyzer's instruction.

7.0 RESULTS

Cell Count 1018
Viable Cell Count 0.0
Viability (%) 0.0
Total Cells / ml (x 1.0E6) 1.04
Viable Cells / ml (x 1.0E6) 0.0
Average Diameter (µm) 8.92
Average Circularity 0.96
Images 50
Averages Cells / Image 20.4
Average Background Intensity 204

8.0 LIMITATIONS

Should any of our materials fail to perform to our specifications, we will be pleased to provide replacements or return the purchase price. We solicit your inquiries concerning all needs for life sciences work. The information given in this bulletin is to the best of our knowledge accurate, but no warranty is expressed or implied. It is the user's responsibility to determine the suitability for their own use of the products described herein, and since conditions of use are beyond our control, we disclaim all liability with respect to the use of any material supplied by us. Nothing

contained herein shall be construed as a recommendation to use any product or to practice any process in violation of any law or any government regulation.

9.0 REFERENCES

Polysciences, Inc. Technical Data Sheet 734.
Cat. # Description Size: 24627 ViaCheck™ Concentration Control (1 x 10^6) 20ml