Moxi Z Cell Counter – Cell Sample Preparation Overview

1. ***Media Selection***
    - ***a.*** The Moxi Z is designed to run with Phosphate Buffered Saline (PBS). Using osmolarity as a coarse estimator of conductivity: ~300 mOsm is preferred. However, most traditional laboratory formulations of physiological tonicity are sufficient for normal particle mode (e.g. HBSS, and MEM). Small Particle Mode (SPM) should be run with PBS.
    - ***b.*** It is preferable that the media be free of particulate matter that could interfere with the counts of interest. In this regard, sterile solutions that have been filtered at .2 micron are ideal.
    - ***c.*** Solutions with less-conductive additives (e.g. 10% FBS or DMSO) are satisfactory for normal mode but might result in a slight increase in reported diameter due to the additive-induced conductivity change of the solution.

2. ***Generate a single cell suspension of your cell sample in buffer of physiological tonicity (i.e. PBS):***
    a. *Adherent Cells*
        i. Detach with suitable dissociation agent in physiological buffer (e.g. StemPro Accutase or Accumax)
        ii. Pipette-triturate detached cell sample to ensure proper cell dissociation
    b. *Suspension Cultures*
        i. Triturate sample (if necessary) to break apart cell clusters
        ii. For strongly clustered cells:
            1. Pellet cells (e.g. 250 x g for 5 minutes)
            2. Re-suspend cells in small quantity (e.g. ~4 ml) of dissociation agent (e.g. Accutase)
            3. Let stand at room temperature for 10-30minutes
            4. Pipette-triturate to disrupt cell clusters
    c. *Primary Cultures*
        i. Ensure harvested cells are properly dissociated into a single-cell suspension
        ii. Eliminate red blood cell (RBC) contamination. (Density gradient, e.g. Ficoll or Magnetic bead purifications are recommended)
        iii. Remove larger particulate by pipetting sample through a cell strainer (ideally a 40 micron strainer). Incubate at 37°C for several minutes

3. ***Dilute the single cell suspension as appropriate based on the concentration range of the cassette type (Type M or Type S) being used:***
    - Type M cassette: 3,000 – 500,000 cells/ml *(If you don't have a ballpark starting cell density, we suggest a first run with a 10-20x dilution using Orflo Diluent or PBS).*
    - Type S cassette: 3,000 – 1,750,000 cells/ml *(If you don't have a ballpark starting cell density, we suggest a first run with a 5-10x dilution using Orflo Diluent or PBS).*

4. ***Mix Sample –*** invert tube a several (10x+ recommended) times before pipetting sample to ensure cell homogeneity. Note: Vortexing and shaking are not efficient approaches for dispersing cells.

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