Development of a novel method to assess primary hepatocyte concentration and viability

INTRODUCTION

Cellometer Vision incorporates image based cell counting and fluorescence detection in a compact and easy-to-use instrument. With dual fluorescence detection capabilities, Cellometer Vision is an ideal solution for many complex cell population characterization assays such as reliably counting and determining viability of primary hepatocytes.

Reliable concentration and viability data of primary Hepatocytes is critical for accurate analysis of compound toxicity in vitro. Due to hepatocytes’ variable morphology, fragile nature and tendency to clump, traditional manual counting methods are time consuming and the subjectivity from operator-to-operator can cause inconsistent results.

Nexcelom’s new method incorporates staining primary hepatocytes with a ready-to-use fluorescent dual staining solution that stains live cells with acridine orange, and dead cells with propidium iodide and then loading 20µL of labeled sample into a disposable counting chamber for analysis. Since the counting chamber is disposable, no washing is required between samples, and the risk of cross contamination is eliminated.

Fluorescent images of the stained cells are captured and using proprietary algorithms, Cellometer Vision’s robust operating software accurately analyzes cell images to generate live cell count, concentration & viability percentage. Total analysis time typically takes less than 60 seconds. Cell images and all analysis data, including cell size distribution histograms, can be instantly saved for documentation. Data can also be easily exported to Microsoft Excel spreadsheets for further analysis.
METHOD

Treat cell sample with Nexcelom’s Fluorescence Dual Staining Solution:
1. Take 20 µl of freshly isolated hepatocyte sample or freeze-thaw cryopreserved cell sample in a small microtube.
2. Apply 20 µl of ready-to-use dual staining solution (acridine orange/propidium iodide cocktail).
3. Gently mix. Sample is ready to count.

Running Assay:
1. Load 20 µl of labeled sample into the Cellometer disposable counting chamber.
2. Insert chamber into Cellometer Vision.
3. Select assay from drop-down menu & enter Sample ID
4. Preview cell images and click ‘Count’ to begin analyzing sample.
5. Review images and counting results.
6. Save or Export images and/or report data.

RESULTS

AO stained live hepatocytes are clearly visible in the fluorescence image obtained from Filter Set 101 (Figure 1). The software indicates counted cells with a green circle (enlarged to show detail) while ignoring cellular debris. The software also can recognize and discretely count clumpy cells. PI stained dead cells are visible in the image obtained from Filter Set 202 (Figure 2). The software then accurately calculates total cell count, concentration and viability (below). By using this method, live and dead cells are clearly distinguished and automatically counted for improved accuracy. By combining a ready to use staining solution and imaging based system results can be obtained much easier and faster compared to other methods.

CELLOMETER Vision Trio SPECIFICATIONS:

| Imaging Modes: Brightfield & 2 Fluorescence Channels |
| Filter Set 101: Excitation/Emission Peak: 475nm/535nm |
| Filter Set 202: Excitation/Emission Peak: 525nm/595nm |
| Dimensions: 6” x 8.5” x 14” (15cm x 22cm x 36cm) |
| Weight: 25lbs (11kg) |
| PC Specs: WinXP/1.8GHz/1GB RAM/laptop included |

To learn more or request a demo, call: (978)327-5340 email: info@nexcelom.com or visit: www.nexcelom.com