



Hybridoma Cell Analyses Using the Beckman Coulter® Vi-CELL™

VI-CELL XR™
Part of the Cell Lab family



Hybridoma Cells

Hybridoma cells are developed in culture by a successful fusion of a spleen lymphocyte and a myeloma cell. The fused hybrid cells survive culture in a selective media such as HAT; whereas the other cells die. Hybridoma cells produce monoclonal antibodies used in both research and disease therapy particularly cancer.

Equipment Used

Beckman Coulter,
Vi-CELL XR
Validated Vi-CELL XR reagent pack

Instrument Settings

The Vi-CELL XR provides the high resolution necessary for accurate results on hybridoma and other cell types. The Vi-CELL software includes the optimum parameters for accurate analyses of a Hybridoma Cell Type which the user may select by a mouse click. The instrument settings for hybridoma cells are the following:

Min. Size (µm) = 7	Cell Brightness = 85
Max. Size (µm) = 50	Cell Sharpness = 100
Number of Images = 50	Viable Cell Spot Brightness = 75
Aspirate Cycle = 1	Viable Cell Spot Area = 5
Trypan Blue Mixing Cycle = 3	Min. Circularity = 0
	Decluster Degree = Low

Results

The results reported by the Vi-CELL are shown in the Results Section of Figure 1. Hybridoma viability and concentration were 43.9% and 1.21×10^6 cells/ml. In addition, the Vi-CELL reports viable cell concentration, mean cell diameter, size distribution, and cellular circularity. The cell images may be archived for future re-analyses.

Conclusion

The Beckman Coulter Vi-CELL automates the standard trypan blue manual cell viability assay. The instrument consistently provides accurate, objective results by removing the subjective variables inherent in the manual method.



Figure 1. Viable cells are circled in green and non-viable in red.

THE VI-CELL

The Vi-CELL automates the widely accepted Trypan Blue Dye Exclusion method. The Vi-CELL combines the state of the art in imaging technology, proprietary algorithm and fluidics management. At the heart of the Vi-CELL is the customized liquid handling system. This system, which allows sample aspiration, reagent handling and subsequent instrument cleaning, is fully automated. Once the cellular suspension has been aspirated and mixed with the trypan blue dye, it is pumped to the flow cell for imaging. The Vi-CELL can analyze up to 100 images for a given analysis increasing total volume from 15 to 30 times over the manual method with result in a less than 2.5 minutes.

MANUAL TRYPAN BLUE DYE EXCLUSION METHOD

As mentioned, the standard method for measuring cell viability is the Trypan Blue Dye Exclusion method. Trypan blue stain (0.4%) is mixed with an equal volume of cells. Viable cells, given their intact membranes, exclude the trypan blue stain; non-viable cells, membrane permeable, stain dark blue. The manual method, however, requires a technician, using a hemacytometer and microscope, to enumerate both stained and unstained cells and manually calculate the percent viability. In addition to being labor intensive, this technique has substantial accuracy error due to its subjective nature.

- Prepare**
 - Automated liquid handling
 - Automated lysing
 - General purpose centrifugation
 - High performance centrifugation
 - Ultracentrifugation
- Identify**
 - Automated fluorescence microscopy
 - Cell counting
 - Cell markers
 - Cell viability analysis
 - Flow cytometry
 - Monoclonal antibodies
- Probe**
 - Automated liquid handling
 - Flow cytometry
 - Microarray technology
 - Monoclonal antibodies
 - Signal transduction assays
- Sort**
 - Cell sorters
 - Micro-piezo electric tips
 - Reagents (various)
- Evaluate**
 - Monoclonal antibodies
 - Multi-mode plate reading
 - Genomics solutions
 - Proteomics solutions
 - Software informatics
- Diagnose**
 - Automated liquid handling
 - Flow cytometry
 - Immunoassays
 - Monoclonal antibodies
 - Software algorithms

VI-CELL TECHNICAL SPECIFICATIONS

INSTRUMENT FUNCTION:

Concentration Range:
 5×10^4 to 1×10^7 cells / mL
 *Counting Accuracy: $\pm 6\%$

OPERATING SYSTEM:

Windows® 98
 Windows® 2000
 Windows® XP

INSTRUMENT TYPE:

Video imaging through a quartz flow cell

POWER REQUIREMENTS:

Power 50 watts
 (65 Watts Max.)
 Voltages 100V, 120V,
 220V or 240V 50/60 Hz

TEMPERATURE:

10° to 40° C (50° to 104° F)

WEIGHT:

11.3kg (25lb)

UNIT DIMENSIONS:

44.5cm (17.5") height
 38cm (15") width
 41cm (16") depth

VI-CELL SERIES

	PN	AUTO SAMPLE	SIZE RANGE (µm)	SAMPLE VOLUME (mL)	ANALYSIS TIME (Min)	VIABILITY RANGE	IMAGING TECHNOLOGY
VI-CELL XR	383556	Yes	2-70	0.5	<2.5	0-100	Auto-focus routine Firewire Camera 1394 X 1040 CCD array
VI-CELL AS	6605769	Yes	5-70	1.0	<3.5	0-100	Manual focus routine Image frame grabber 640 X 480 CCD array
VI-CELL S	383080	No	5-70	1.0	<3.5	0-100	Manual focus routine Image frame grabber 640 X 480 CCD array
VI-CELL XR QUAD PACK	383722						
VI-CELL AS, S QUAD PACK	383198						
VI-CELL CONCENTRATION CONTROL	175478						
VI-CELL FOCUS CONTROL	175474						



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