BECKMAN COULTER
Life Sciences

Vi-CELL BLU FAST Mode Option

The Vi-CELL BLU instrument introduced a new plate reader capability to the Vi-CELL family of cell analyzers. The plate option gives the user the ability to load up to 96 samples or a combination of samples and controls onto the instrument for continuous operation.

However running so many samples will result in a significantly long run time, approximately 3 hrs total for a full 96 well plate. This may raise some concerns about occupying an instrument for such a long time or viability drift in cells that are not particularly stable outside the incubator environment. To help mitigate these concerns we have equipped the Vi-CELL BLU with an optional FAST Mode of operation which can be used in either plate or carousel configuration to significantly reduce sample to sample operation times.

Wash Mode	Volume Needed	Sample Run Time Interval 100 images, ~2x10^cells/ml (mm:ss)	Full Plate time 96 samples 100 images, ~2x10^cells/ml (h:mm:ss)
Normal Wash	200 µL	<130 seconds Typical analysis time: Normal mode 110 seconds	Estimated 3:28:00 Typical: 2:56:00
Fast Wash	170 µL	<90 seconds Typical analysis time: 80 seconds	Estimated 2:24:00 Typical: 2:08:00

The FAST Mode shortens the wash cycle duration saving approximately 30% on sample run time. The tradeoff is a potential increased risk of carry over between samples since the washing is less thorough than normal mode. This tradeoff may be acceptable depending on sample type and desired run time.

Please note also that prolonged use of Fast Mode may result in increased build-up of trypan blue and proteinaceous material in the fluidics path of the instrument, so regular decontamination runs are recommended.

Setting Up Fast Mode

Wash mode is a configurable option during sample logging or set as the default in the Run Setting preferences screen. It can either be chosen in the sample setup bar which would allow multiple samples to be logged using the same settings, or it can be changed once the sample is in the queue list. For more instructions please consult the user manual.

Assessing Run–Run Carry Over

The basic scheme for assessing carryover is to alternate a cell or bead sample with blank buffer samples, in either a plate or carousel and to run either in Normal or Fast Wash mode. An example of such a run is shown below.

Sample ID	Wash	Sample Type	Cell count
…	…	…	…
Beads–L07–norm.015	Normal Wash	L5 Bead	11903
Blanks–L07–norm.016	Normal Wash	Blank	21
Beads–L07–norm.017	Normal Wash	L5 Bead	12061
Blanks–L07–norm.018	Normal Wash	Blank	5
Beads–L07–norm.019	Normal Wash	L5 Bead	11444
Blanks–L07–norm.020	Normal Wash	Blank	26
Beads–L07–fast.001	Fast Wash	L5 Bead	11717
Blanks–L07–fast.002	Fast Wash	Blank	24
Beads–L07–fast.003	Fast Wash	L5 Bead	11368
Blanks–L07–fast.004	Fast Wash	Blank	28
…	…	…	…

L5 size standard beads (6602794 lot 9012057F) were used as a general control due to their nominal size and approximate particle density of ~4 x 10^6 beads/mL. The instrument was run using a cell profile set up for L5 beads based on the default L10 bead profile.

CHO cells are a standard cell type that was used to determine carryover when using a biological sample. Absolute concentration was approximately 3 x 10^6 cells/mL but this is not a critical parameter as the main investigation is the relative number of cells between the normal and blank samples. Cells were analyzed using standard Mammalian cell type profile.

Particle concentration was not critical here but was used an indicator of expected performance. Particle counts between sample and blank were recorded. Residual carry over was determined by the number of beads appearing in the blank samples. Random analysis of captured images was also conducted to verify particles scored in blanks were bead and not some other debris.

Blank samples have highly variable count numbers as expected when running a sample with no particles. This causes some issues with the internal calculations so average particle counts are the most consistent parameter to use.

Results

Instrument Settings for Analysis

Cell type	BCI L5 Beads	Mammalian
Minimum Diameter (µm)	2	6
Maximum Diameter (µm)	10	30
Images	100	100
Cell sharpness	22	7
Minimum circularity	0.75	0.1
Decluster degree	Medium	Medium
Aspiration cycles	3	3
Viable spot brightness (%)	50	55
Viable spot area (%)	1	5
Mixing cycles	3	3

The results below show multiple runs on both carousel and plate sample loading. The data are presented as paired results showing the average of the sample count and the average of the carry over count recorded between samples.

L5 Beads

Run	Wash Mode	L5 Beads Count	Carryover Bead Count	% Carryover	# Sample pairs
1	Normal Wash	10629	108	1.01%	20
2	Normal Wash	11784	11	0.10%	26
3	Normal Wash	11414	164	1.44%	60
4	Normal Wash	11862	236	1.99%	60
5	Normal Wash	11326	161	1.42%	40
1	Fast Wash	9392	226	2.40%	20
2	Fast Wash	11147	38	0.34%	54

CHO Cells

Run	Wash Mode	CHO Cell Count	Carryover Cell Count	% Carryover	# Samples pairs
1	Normal Wash	3939	3	0.07%	38
2	Normal Wash	5546	9	0.16%	48
3	Normal Wash	7986	3	0.04%	26
4	Normal Wash	5310	27	0.51%	24
1	Fast Wash	3952	5	0.13%	38
2	Fast Wash	5281	23	0.44%	36
3	Fast Wash	8595	21	0.24%	24

Conclusions

A T-test analysis between normal and fast wash residual bead counts in the blank samples gives a p value of 0.986 indicating no statistical difference between operation modes in terms of carry over between samples. It is therefore possible operate the instrument in either mode and have confidence that the sample to sample carryover will be negligible.

It should be noted that the degree of variability in the blank samples can be extremely high due to so few particles being counted but in all test cases the percentage carry over was always well below 1%.

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