BECKMAN COULTER Life Sciences

Automated Cell Plating and Growth Assays

Summary

- Automated dilution and plating of CHO cells
- Confirmed plating consistency with imaging assays
- Automated XTT assay to determine linearity to relative cell numbers

Cellular applications require consistent plating of cells and sample preparation so that cellular changes can be accurately attributed to experimental treatments. Confirmation of consistent plating can be achieved through imaging wells or through surrogate assays such as XTT cell viability kits. These assays can also be used to detect differences in cell growth, such as in cell toxicity or media optimization screens. Automation can improve the consistency of cell plating and assay sample preparation and reduce the opportunity for errors. Here we show how the Biomek i5 Workstation (Figure 1) was used to serially dilute a cell suspension, plate the resulting cells and prepare them for analysis by XTT.

It is essential that cell cultures and assays are maintained in sterile conditions to prevent microorganism contamination. A HEPA filter (Figure 1) can be added to the enclosed Biomek i5 Workstation and coupled with sterile pipette tips to maintain sterile techniques during cellular manipulations on the deck.

Figure 1. Biomek i5 Span-8 Workstation with HEPA-filtered enclosures

A suspension of Chinese hamster ovary (CHO) cells ($2X10^5$ cells/mL) was added to a well of a deepwell plate and the Biomek i5 performed a 1:2 dilution down the column. 100 µL of the resulting suspensions was plated in triplicate wells. Pipetting was optimized to prevent the introduction of bubbles into the well to ensure even distribution of cells and gas exchange throughout the well. Cells were incubated at 37°C, 5% CO_2 for 4 hours prior to analyzing cell counts on a SpectraMax® i3x Multi-Mode platform with SpectraMax MiniMax 300 Imaging Cytometer. Figure 2A shows the average cell count for each dilution and Figure 2B plots the resulting cell counts against the predicted plating number. The high R^2 value (0.9996) indicates consistent dilution and cell plating.

A

Cells Plated	Avg. Count	CV
20000	6290	4.1%
10000	3264	4.5%
5000	1662	4.5%
2500	888	2.4%
1250	483	7.8%
625	237	1.1%
313	136	3.0%

B

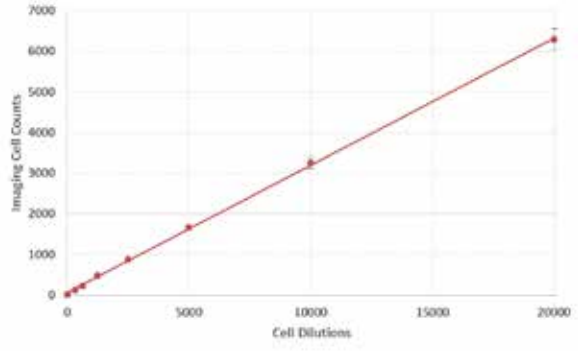

Figure 2. Imaging Cell Count. Four brightfield images were acquired per well for triplicate wells at each dilution on a SpectraMax MiniMax cytometer. A) Average cell counts and coefficient of variability for triplicate wells. B) Average cell count plotted against plated cells. Error bars show standard deviations of triplicates. Excellent linearity (R2 = 0.9996) indicates consistent serial dilution and plating.

50 µL XTT reagent was added to the triplicate wells and returned to the incubator for 4 hours. Absorbance values at 475 nm were acquired on the SpectraMax i3x Multi-Mode platform and used as a measurement for the total cells per well. Figure 3A shows the consistency of cell and reagent addition across triplicate values (CV < 2%) and Figure 3B shows the linearity (R^2 = 0.9991) confirms the consistent cell dilution seen by imaging.

A

Cells Plated	Avg. Absorbance	CV
20000	1.712	1.9%
10000	0.975	1.7%
5000	0.630	1.6%
2500	0.454	1.9%
1250	0.378	0.5%
625	0.342	0.8%
313	0.325	0.6%
0	0.310	1.4%

B

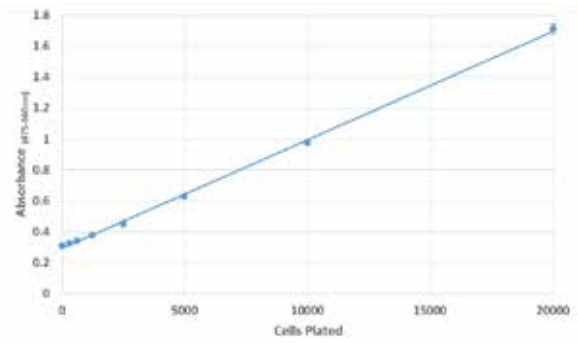

Figure 3. XTT Assay. XTT reagent was added to cells four hours after plating and incubated for four additional hours. A) Average absorbance readings and CV for triplicate wells. B) Plot of absorbance vs. plated cells (error bars = standard deviation). Linearity (R^2 = 0.9991) and low variability (CVs <2%) indicate high reliability in the automated XTT assay.

This work demonstrates the ability to reliably plate cells and assay their growth by XTT analysis with excellent consistency. For higher throughput applications, such as cell-based screens, one could utilize a Biomek i-Series instrument with a Multichannel head and/or integrate the incubator and analyzers needed for a complete workflow.

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