Celigo-based cell-counting and viability assay for mammalian cells

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Henning Gram Hansen
CHO Cell Line Engineering and Design, NNF-CfB, Technical University of Denmark
hgra@biosustain.dtu.dk*Corresponding Author*

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Abstract

Accurate assays for counting mammalian cells are important tools when performing experiments in cell biology. In general, commercially available cell-counting assays suffer from low throughput and high costs of consumables. Here, we describe a cost-efficient Hoechst and propidium iodide (PI)-staining protocol that accurately counts the number of viable mammalian cells in 96-well microplates based on image cytometry on a Celígo® Image Cytometer. Hands-on time for one microplate is less than 20 minutes and the entire procedure including incubation takes approximately 1 hour. Since all steps prior to image cytometry analysis involve liquid handling and since the Celígo® Image Cytometer is automation-friendly, this protocol has the potential to be integrated into automated liquid handling workflows.

Reagents

96-Well Greiner™ 655090 Plate

1000xPI (sterile-filtered 0.4 mg/mL propidium iodide in dH2O, Sigma, cat# P4170)

2500xHoechst (‘Hoechst 33342’ 12.3 mg/mL 20 mM, ThermoFischer Scientific, cat# 62249)

CD CHO Medium (ThermoFischer Scientific, cat# 10743029)

200 mM L-Glutamine (Lonza, cat# 17-605)

CHO-S suspension cells - the protocol can probably also be used for other mammalian cells (adherent and suspension cells)

Equipment

Celígo® S Cell Cytometer (Nexcelom)

Procedure

Make the staining solution:

o 8 µM Hoechst, 0.4 µg/mL PI in complete media (CD CHO + 8 mM L-Glutamine)

Pipet 3 uL cell suspension to wells in the 96-well plate (a multi-channel pipette can be used)

o Remember to resuspend cells before pipetting

Add 200 uL staining solution to each well (a multi-channel pipette can be used)

Pipet 150 uL up and down three times in each well (a multi-channel pipette can be used)

o Aspirate at a steady pace

o Dispense at a relatively fast pace to ensure proper mixing

o Perform each pipetting step at different spots on the bottom of the well

Incubate at room temperature in the dark for 40 min to allow the cells to sediment and for the Hoechst stain to increase in intensity

o Hoechst and PI stains are stable for at least two hours.

-Scan wells on the Celigo using the mask (blue channel; Hoechst) and target 1 (red channel; PI) application.

o Use fixed exposure time and gain settings for the red channel.

Use a fixed gate that divide PI positive and negative cells

o Make sure to have healthy cells \(wt/parental/host cell line) in one well so you know that your PI-gate is divic

Notes:

Equations:

Viability="PI-negative cells in the well"/"total cells in the well"

VCD="total cells in the well"**1000/3**Viability

Working range for healthy as well as stressed/dying (long-term cultures) cells:

2e5-2e6 total cells/mL

The wide linear dynamic range in the paper only applies to healthy cells.

Figures

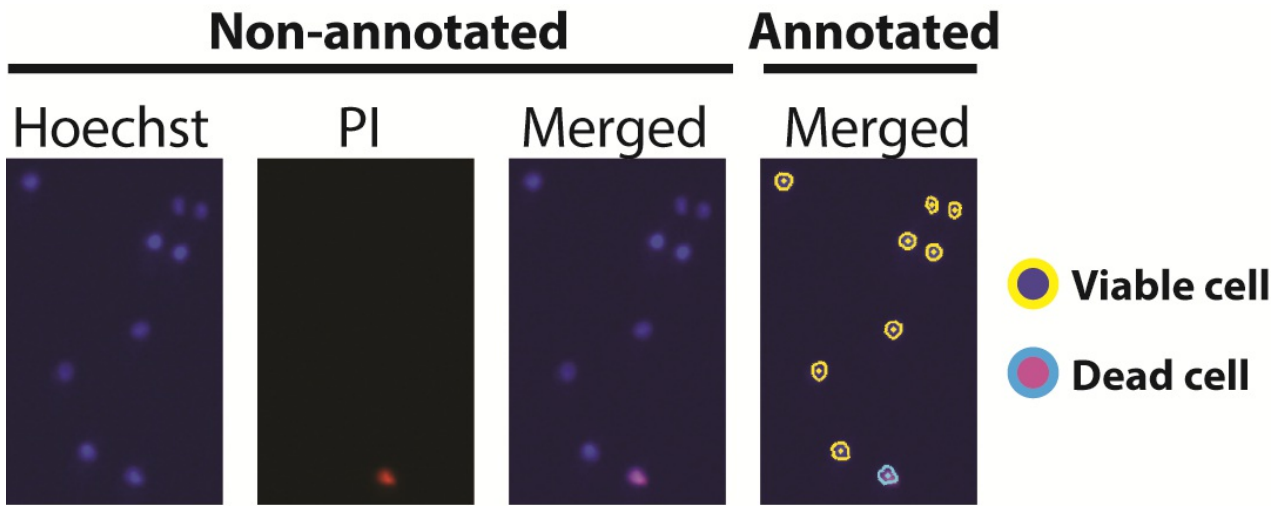

Figure 1

Celigo images Celigo images of Hoechst and PI-stained cells

Versatile microscale screening platform for improving recombinant protein productivity in Chinese hamster ovary cells

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