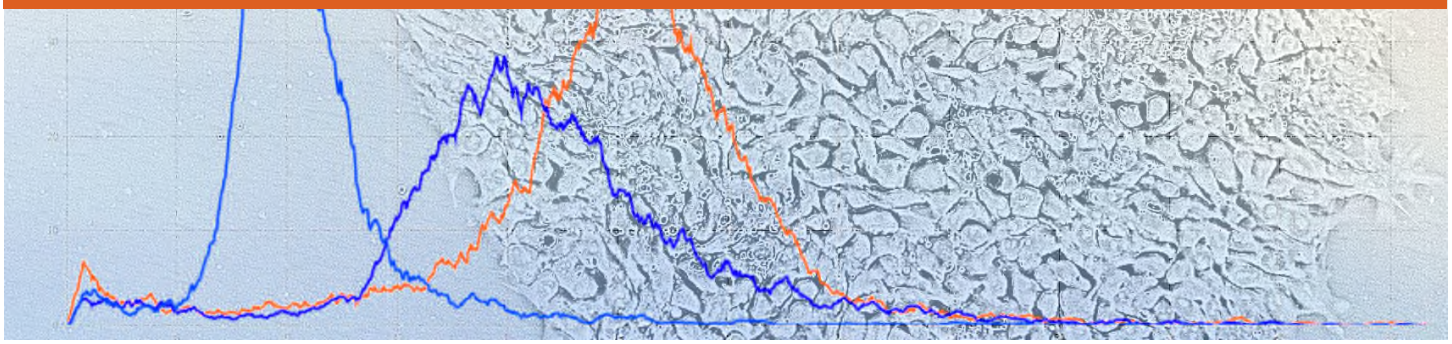
OMNI Life Science

Application Note

iPS Cell Propagation: Fast & Easy Check for Viability and Aggregation

Precise and reproducible Quality Control of iPSCs with CASY



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Introduction

Availability of induced pluripotent stem cells (iPSCs) provides unique opportunities for Biomedical research. Currently, the iPSCs are propagated using enzymatic and non-enzymatic approaches. For optimal useability of the cells, it is particularly important to gain a clear picture of the iPSCs regarding their quality. Therefore, the feasibility of the CASY cell analyzer was evaluated regarding iPSC viability and proliferation during cell propagation using Accutase and EDTA treatment of iPSCs. We found single iPS cells after Accutase treatment, while EDTA treatment developed clusters.

Methods

Stem Cells

Human induced pluripotent stem cells were harvested in parallel from adherent cultures by Accutase and EDTA method. For the analysis, 100µl of cell solution were mixed with 10ml of CASYton in a CASYcup.

CASY Analysis

Diluted cells were analyzed by CASY cell analyzer using the following settings (program for iPSC):

- *150 µm capillary*
- *400 µL sample volume, triplicate measurement*
- *x-scale set to 50 µm*
- *Range for dead cells 7.76 to 12.32 µm*
- *Range for viable cells 13.33 to 50 µm*
- *Aggregation correction: manual 2690 fl*

Protocols

EDTA detachment procedure for iPSCs (cluster) *[1]*

1. *For 1 well/6-well plate, equilibrate 2 mL 0.5mM EDTA solution to room temperature before use.*
2. *Prepare a CASYcup with 10 mL CASYton solution.*
3. *Wash the cells with DPBS without Ca^{2+} and Mg^{2+}.*
4. *Pipette 2 mL 0.5 mM EDTA solution into the well and incubate the cells for 4 min at room temperature under the laminar flow.*
5. *Check the detachment process of the cells optically under the microscope: after 4 min the cells usually start to detach, but tend to stay cluster.*
6. *Carefully aspirate the EDTA solution.*
7. *Carefully detach the cells from the well surface by rinsing the surface of the well with 10 mL culture medium and transfer the solution to a 15 ml Falcon tube.*
8. *Carefully resuspend the cells and transfer 100 µl of the cell suspension into the CASYcup containing 10 mL CASYton solution from step 2.*
9. *Immediately analyze using CASY program for iPSC.*

Accutase detachment procedure for iPSC (single cells) *[2]*

1. *For 1 well/6-well plate, equilibrate 1 mL Accutase solution to room temperature before use, do not warm to 37 °C (activity of the enzyme decreases rapidly at 37 °C).*
2. *Prepare a CASYcup with 10 mL CASYton solution.*
3. *Aspirate the medium.*
4. *Wash the cells with DPBS without Ca^{2+} and Mg^{2+}.*
5. *Pipette 1mL Accutase into the well and incubate the cells for 7 min at 37 °C in the incubator.*
6. *Check the detachment process of the cells optically under the microscope: after 5 min the cells usually start to detach, but tend to stay cluster. It might be beneficial to incubate them for additional 2 min at 37 °C to achieve a single cell suspension. Do not incubate longer than 10 min.*
7. *Carefully resuspend the cells and transfer 100 µl of the cell suspension into the CASYcup containing 10 mL CASYton solution from step 2.*
8. *Immediately analyze using CASY program for iPSC.*

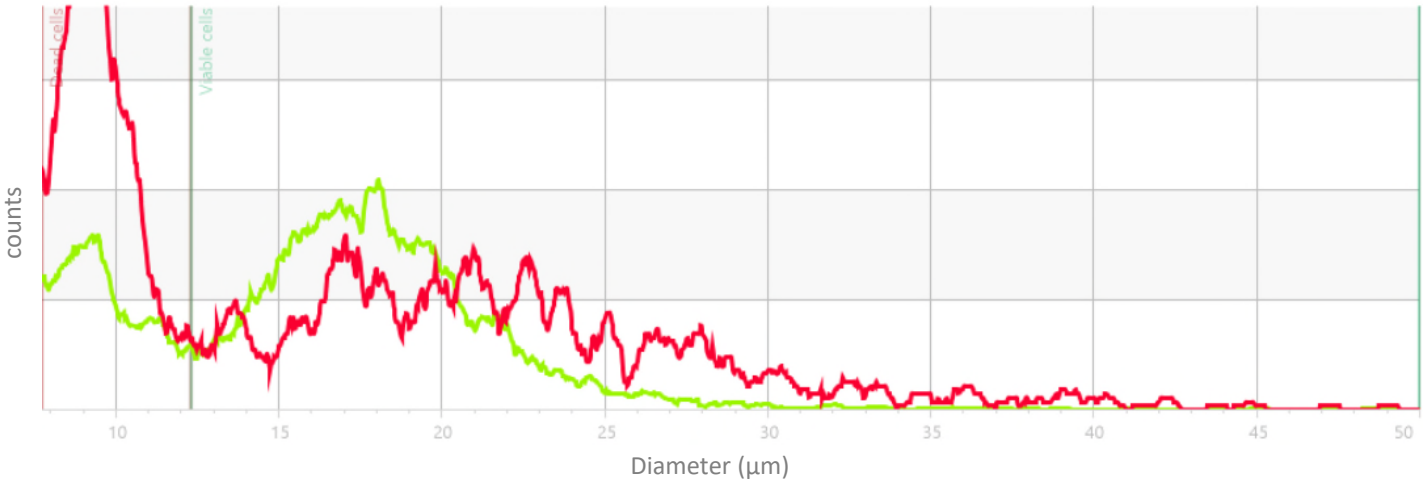

Table 1. **CASY analysis result: Cell viability and cell aggregation factor after using Accutase or EDTA dissociation method.**

Sample (Fig.1)	dead cells (counts/mL)	viable cells (counts/mL)	% viability	aggr. factor
hiPSCs EDTA (red)	8.66×10^4	3.28×10^5	79	2.7
hiPSCs Accutase (green)	7.28×10^4	2.98×10^5	80	1.4

Fig. 1: **Size distribution of human iPSCs.** Representative CASY cell analyzer data demonstrating size-based discrimination of iPSCs harvested with Accutase (green) and with EDTA (red). As shown, the cells appear mainly as single cells with less remaining aggregates (green), while dissociated with EDTA the cells are predominantly restricted to clusters (red).

Results

Accutase: Less Aggregates

From size distribution analysis with CASY, Accutase-harvested cells appeared with one single peak indicating less aggregates in the preparation, confirmed by calculation of the aggregation factor (table 1) performed by CASY.

EDTA: More Cell Clusters

The jagged line of size distribution in figure 1 clearly indicates residual cell aggregates after EDTA treatment, also expressed as an elevated aggregaton factor in CASY statistical analysis (table 1).

References

1. Beers et al.. Passaging and colony expansion of human pluripotent stem cells by enzyme-free dissociation in chemically defined culture conditions. Nat. Protoc. 2012 Nov;7(11):2029-40. doi:10.1038/nprot.2012.130
2. Bershteyn M et al.. Human iPSC-Derived Cerebral Organoids Model Cellular Features of Lissencephaly and Reveal Prolonged Mitosis of Outer Radial Glia. Cell Stem Cell. 2017 Apr 6;20(4):435-449.e4. doi: 10.1016/j.stem.2016.12.007.

Conclusion

CASY cell analyzer represents a powerful tool enabling a precise, robust and reproducible analysis of iPSCs. This analysis showed no significant difference as to the obtained average **viability rates** in Accutase (80,4%) and EDTA (79,1%) harvesting method.

However, depending on treatment, the **iPSCs are distinguishable in size and morphology**: EDTA treated cells formed clusters what immediately became apparent in the size distribution plot and aggregation factor calculation during sample analysis.

This study provided real time information on iPSC proliferation, viability and aggregation state during preparation of the cells – valuable parameters for **iPSC quality control** as a basis for subsequent processing of cells.

Request more information

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