# Passaging methods for hPSCs under feeder-free conditions

## Introduction

From basic science to translational medicine, human pluripotent stem cells (hPSCs) are a valuable yet delicate resource. Unlike their robust mouse counterparts, hPSCs are highly susceptible to cell death and differentiation. Research shows that hPSC culture conditions and dissociation methodology significantly impact cellular characteristics (i.e. growth rate), and potential in downstream applications (i.e. differentiation capacity). In addition, the long-term goal of therapeutic stem cell use requires special consideration, including culture scalability and avoidance of xenogenic-factors.

hPSCs have shown to grow well when supported by paracrine factors previously found either in fetal bovine serum, or secreted from mitotically inactive mouse or human fibroblasts, known as a "feeder layer"[1,2]. However, variability in these animal-derived products necessitated the development of feeder-free culture systems, which rely on chemically defined basal media, recombinant growth factors, and components of extracellular matrix to support growth and adhesion of hPSCs[3]. Matrigel™ is a widely used and referenced hPSC culture substrate for feeder-free culture, however it contains xenogenic factors and is not suitable as a clinical-grade cell culture compounds[4,5]. Other products of solely human origin like purified recombinant matrix proteins (i.e. Vitronectin ACF, BI, cat.# 05-754-0002, and LaminStem™ 521, BI, cat.# 05-753-1F) are excellent alternatives for feeder-free hPSC culture.

To facilitate nutrient availability, continued growth, and expansion of hPSC lines under feeder-free conditions, large colonies require routine dissociation and replating. This process, known as passaging or subculturing, can be performed through a variety of well-defined methods.
Large aggregate passaging utilizes manual dissociation, alone or in combination with enzymes, such as dispase and collagenase. Small aggregate passaging utilizes non-enzymatic solutions like EDTA, while trypsin-based products or non-enzymatic reagents can be used in combination with apoptosis inhibitors to facilitate single cell dissociation. Choice of dissociation methodology often varies, dependent on downstream experiments. Factors including length of experiment, scalability, and cost, must be balanced with overall culture health.

## Passaging as Large Aggregates

Passaging of hPSC as large aggregates was once considered standard practice for hPSC culture. Although cellular damage and death occur (<50% viability), this method maintains healthy cultures, preserving normal karyotype, pluripotency markers, and differentiation capacity for numerous passages. To generate large aggregates, a skilled technician may manually remove differentiated colonies by micro-dissection. Following this, remaining colonies are scored and/or scraped with a glass pipette, and collected via gentle centrifugation. Manual passaging as large aggregates can also be combined with enzymatic dissociation using dispase or collagenase (recommended at a concentration of 1mg/mL in medium) to facilitate colony detachment and increase the yield of viable cells for expansion[7]. While these methods are compatible with Matrigel™, or other protein matrices, they cannot be used on cultures grown in large-scale, multi-layer plates. As such, large aggregate passaging is most commonly used for low-throughput, research experiments. Large aggregates are also poorly suited for transfection experiments, where access to cell surface facilitates efficient transfer of plasmids or small RNAs.

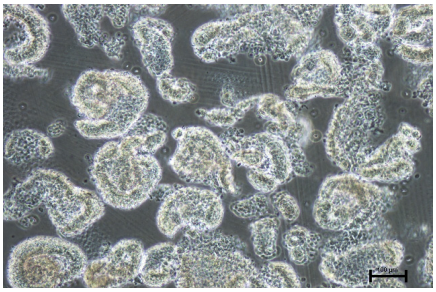

**Figure 1:** hPSCs cultured in NutriStem® hPSC XF medium and passaged as large aggregates (clumps) X100

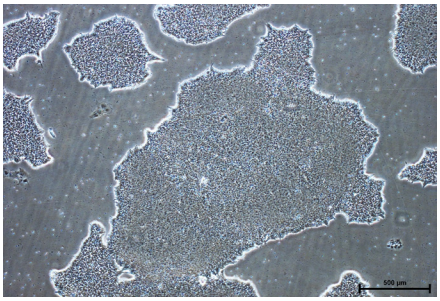

**Figure 2:** Typical recovery of hPSCs from collagenase dissociation (day 3 post passage) x40

## Passaging as Small Aggregates (Enzyme Free Passaging)

In contrast to large aggregate and single cell methods for hPSC dissociation, small aggregate dissociation is a gentle, enzyme-free method of passaging cells grown in feeder-free conditions. 0.5mM EDTA (BI, cat.# 01-862-1B) mediates rapid cell dissociation by chelating calcium and magnesium ions that facilitate cell adhesion. While this method is associated with high cell viability (>60%), the use of high concentrations or extended incubation periods will result in generation of single cells, and apoptosis. Studies suggest aggregates between 50 - 100 µm in size are optimal for maintaining cell-to-cell connection and balancing efficiency of transfection or other experimental methods. Because EDTA has a high affinity for calcium ions, careful timing of dissociation with EDTA is critical.

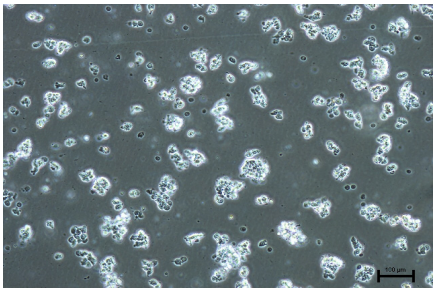

**Figure 3:** hhPSCs cultured in NutriStem® hPSC XF medium and passaged as small aggregates (mini clumps) X40

**Figure 4:** Typical recovery of hESCs from enzyme- free dissociation using 0.5mM EDTA solution (day 3 post passage) x40

## Passaging as Single Cell

With the onset of genetic manipulation of cells, genetic editing and CRISPR/Cas9 applications, single cell passaging is slowly on the rise. The use of completely dissociated single cell suspension for passaging generates a monolayer culture that has advantages of higher culture scalability, rapid expansion, and high efficiency. The introduction of recombinant protein matrices (i.e. LaminStem™ 521, BI, #cat 05-753-1) and recombinant trypsin solutions (i.e. Recombinant Trypsin EDTA Solution, BI, cat.# 03-079-1) now enables the expansion of hPSCs in a monolayer. This enables single cell passaging to be more efficient and viable, without the addition of ROCKi, while maintaining cell integrity and characteristics. With the support from the laminin-521 matrix, cells can be passaged in very low cell densities and can be cultured to high confluence without phenotypic alterations[6]. Laminin-521 also supports efficient clonal culture and is an excellent substrate for iPSC reprogramming. When used with NutriStem® hPSC XF medium, (BI, cat.# 05-100-1A) LaminStem™ 521 has been proven to promote cellular survival and expansion of hPSC after plating from single-cell suspension. When cultured with LaminStem™ 521, the hPSC grow as a monolayer and remain pluripotent without spontaneous differentiation.

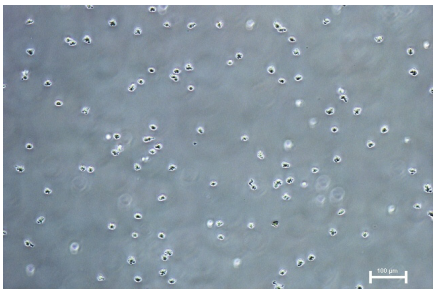

**Figure 5:** hPSCs cultured in NutriStem® hPSC XF medium and passaged as single cells X100

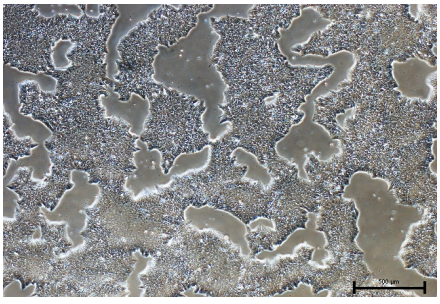

**Figure 6:** Typical recovery of hESCs from single cell passage using Recombinant Trypsin EDTA Solution
(day 4 post passage) x40

## Comparison Table

| Dissociation Method | Large aggregates passaging | Small and large aggregates passaging | Small aggregates passaging | Single cell passaging |
|---|---|---|---|---|
| **Cell Dissociation Reagent** | Collagenase | Accutase | 0.5M EDTA solution | Recombinant Trypsin EDTA Solution |
| **Intended Matrix** | • Matrigel<br>• Feeder | • Matrigel<br>• Feeder | • Matrigel<br>• Vitronectin ACF | Laminstem-521 |
| **Benefits** | Widely referenced and well established dissociation method | Gentle dissociation method | • High cellular viability<br>• Non-enzymatic dissociation reagent<br>• Cost effective<br>• Does not require ROCK inhibitor | • Animal component free dissociation reagent<br>• Does not require ROCK inhibitor |
| **Drawbacks** | Animal-derived dissociation reagent (lot-to-lot variability) | Animal-derived dissociation reagent (lot-to-lot variability) | | |
| **Detach time** | 5-10 min. | 5-45 min. | Time sensitive: only 3-4 min. detach time | Time-sensitive: only 2-4 min. detach time |
| **Recommended culture medium** | NutriStem® hPSC XF Medium | NutriStem® hPSC XF Medium | With Matrigel™: NutriStem® hPSC XF Medium<br>With Vitronectin ACF: NutriStem® V9 XF Medium | NutriStem® hPSC XF Medium |

| Product | Cat.# | Size |
|---|---|---|
| NutriStem® hPSC XF Medium | 05-100-1A | 500mL |
| NutriStem® V9 XF Medium | 05-105-1A | 500mL |
| Vitronectin ACF | 05-754-0002 | 200 µg |
| 0.5M EDTA solution | 01-862-1B | 100mL |

| Product | Cat.# | Size |
|---|---|---|
| Recombinant Trypsin EDTA Solution | 03-079-1B | 100mL |
| Accutase | 03-073-1B | 100mL |
| LaminStem™ 521 | 05-753-1F | 1 mL |

## References


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