# mTeSR™3D

## Suspension Culture Medium for hPSCs

## Introduction

mTeSR™3D is the newest member of the TeSR™ family of media and has been specifically developed for expansion of human pluripotent stem cells (hPSCs) as aggregates in suspension culture. With its suspension-optimized formulation and simplified fed-batch workflow, mTeSR™3D enables scale up to 1 x $10^9$ undifferentiated hPSCs in as little as 2-3 weeks, starting from a single 6-well plate.


## mTeSR™3D and Fed-Batch Culture

mTeSR™3D employs a fed-batch strategy, which eliminates the need for daily medium exchanges, saving you time and medium. The mTeSR™3D kit consists of a Seed Medium and a Feed Medium:

- mTeSR™3D Seed Medium (Basal + 5X Supplement) is used to initiate suspension cultures from 2D cultures or after passaging.
- mTeSR™3D Feed Medium (Supplement A + Supplement B) is used to feed the suspension cultures on the non-passaging days.

## Weekend-free passaging

To save your weekends, the protocol has been optimized with an alternating 3-day/4-day passaging schedule (Fig 1). On day 0, hPSCs are seeded into mTeSR™3D Seed Medium supplemented with 10 µM ROCK inhibitor Y-27632. On subsequent days, only a simple addition of mTeSR™3D Feed Medium is required, with no need for a full medium exchange.

## Advantages

**OPTIMIZED.** Part of the mTeSR™ family, mTeSR™3D is optimized for hPSC suspension culture.

**SIMPLIFIED WORKFLOW.** Fed-batch strategy provides a simplified culture system.

**DEFINED.** Serum-free culture system with no microcarriers or external matrix required.

**SCALE UP.** Easily produce up to 1 x $10^9$ hPSCs in as little as 2-3 weeks.

**VERSATILE.** Compatible with a variety of suspension culture vessels.

**COST EFFECTIVE.** Significant cost savings in both media and labor.

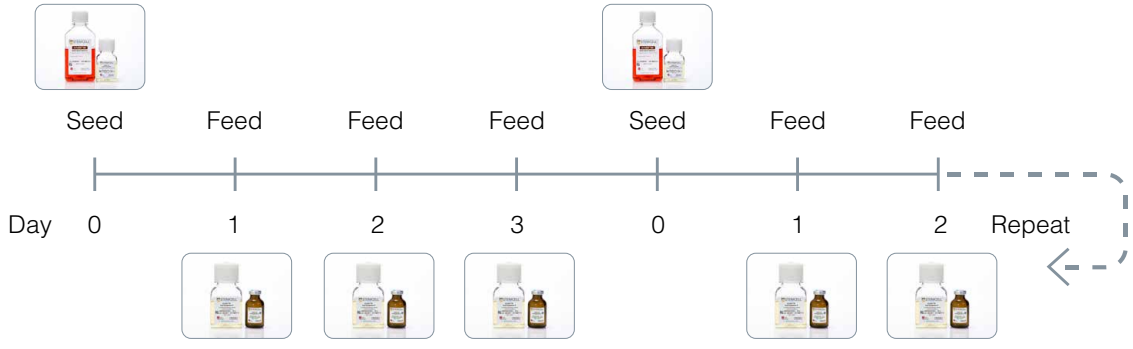


**Figure 1.** mTeSR™3D Employs a Fed-batch Strategy.

Human PSCs expanded in the mTeSR™3D fed-batch culture system have robust growth (Fig 2) and maintain high expression of pluripotency markers (Fig 3). The hPSC aggregates have consistent morphology and are generally uniform in size at the end of each passage (Fig 4). For best results, aggregates should be approximately 350-400 µm by the end of the passage. hPSCs cultured in mTeSR™3D retain trilineage differentiation ability and show robust differentiation to all 3 germ lineages (Fig 5).

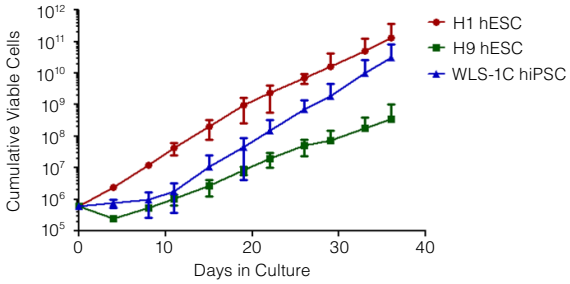


**Figure 2.** Cumulative Growth of hPSCs in mTeSR™3D.

Multiple hPSC cell lines were expanded in mTeSR™3D using fed-batch feeding and clump passaging for 10 passages. All cell lines tested showed robust growth. Growth rate is cell line-dependent. Error bars represent 95% confidence interval.

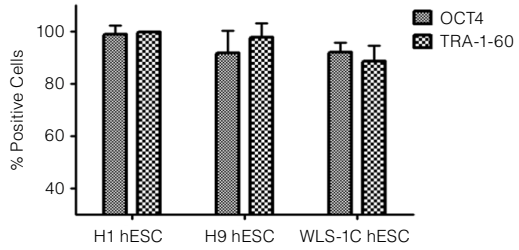


**Figure 3.** Maintenance of Undifferentiated Cell Markers in mTeSR™3D.

hPSCs show retention of undifferentiated cell markers OCT4 and TRA-1-60 after 10 serial passages in mTeSR™3D. Error bars represent 95% confidence interval.

**Figure 4.** Morphology of hPSC Aggregates in mTeSR™3D.

hPSC aggregates of approximately 350-400 µm in size are generated after 3-4 days in mTeSR™3D.

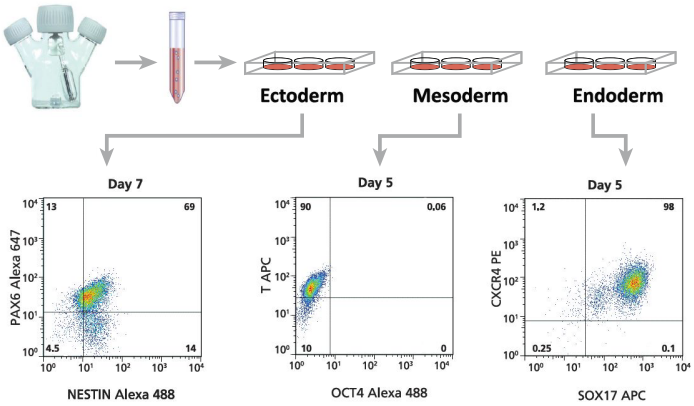


**Figure 5.** Trilineage Differentiation of hPSCs after Multiple Passages in mTeSR™3D.

hPSCs cultured in mTeSR™3D efficiently differentiate to all three germ lineages. The STEMdiff™ Trilineage Differentiation Kit (Cat # 05230) was used to assess the differentiation potential of mTeSR™3D suspension-grown cells. Plots shown are H1 hES cells, similar results are seen with other cell lines tested, including H9, H7 and WLS-1C.

| PRODUCT | DESCRIPTION | CATALOG # |
|---|---|---|
| mTeSR™3D | mTeSR™3D Kit for Suspension Culture of hPSCs includes:<br>• mTeSR™3D Seed Basal Medium, 400 mL<br>• mTeSR™3D Seed 5X Supplement, 100 mL<br>• mTeSR™3D Feed Supplement A, 100 mL<br>• mTeSR™3D Feed Supplement B, 12 mL | 03950 |
| Y-27632 | RHO/ROCK pathway inhibitor | 72302 |

## Global Exclusive Licensing

mTeSR™3D is manufactured and sold under global exclusive license from Accellta for culture medium for hPSCs in suspension under feeder-free, non-adherent conditions.