



TECHNICAL BULLETIN

Transitioning Human Embryonic and Induced Pluripotent Stem Cells from TeSR™2 to mTeSR™1

Introduction

mTeSR™1 and TeSR™2 are standardized media for the feeder-independent maintenance of human embryonic and induced pluripotent stem cells (hESCs and hiPSCs). These are complete, serum-free, defined formulations based on the publications by Ludwig et al.^{1,2} and should be used with BD Matrigel™ hESC-qualified Matrix (BD Catalog #354277) as a substrate. STEMCELL Technologies has pre-qualified each batch of BD Matrigel™ to ensure consistency, reproducibility and reliability in performance.

No adaptation step is required when transitioning cells that have been maintained in TeSR™2 into mTeSR™1. Simply replate hESC or hiPSC clumps into mTeSR™1 at the time of passaging. There are, however, several minor characteristic differences of culturing hESCs and hiPSCs in mTeSR™1, which are highlighted below. Additional information for the passaging of hESCs and hiPSCs in mTeSR™1 is provided in the complimentary technical manual (Catalog #29106) "Maintenance of hESCs and hiPSCs in mTeSR™1 and TeSR™2" available on our website at www.stemcell.com.

Colony Morphology and Growth Kinetics

Colony morphology for hESCs and hiPSCs maintained in mTeSR™1 varies slightly compared to the same cells in TeSR™2. Cells in either media are ready to be passaged when colonies are large, beginning to merge, and have centers that are dense and phase-bright compared to their edges (Figure 1).

FIGURE 1. Morphology of (A) hESC H9 colonies in mTeSR™1 and (B) hiPSC iPS(IMR90)-1 colonies in TeSR™2 that are ready to be passaged.

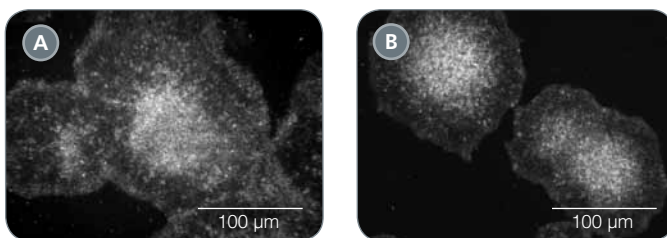
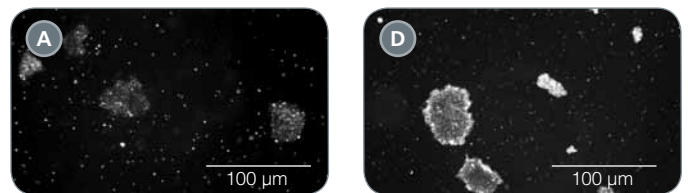


Photo (B) courtesy of Dr. T. Ludwig, WiCell Research Institute

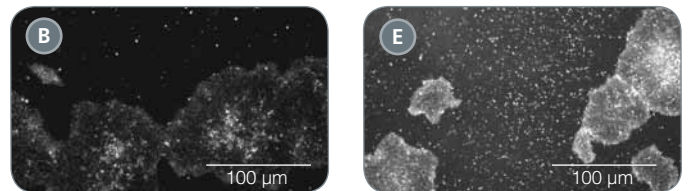
Colonies in mTeSR™1 are less densely packed with cells and will appear more transparent at early time points compared to colonies grown in TeSR™2. The cell density within the colonies increases rapidly in mTeSR™1 after approximately 4 days, at which point colonies will begin to resemble the morphology of

colonies observed in TeSR™2 (Figure 2). Depending on the size and density of seeded aggregates, hESC and hiPSC cultures are usually passaged 5–7 days after seeding in mTeSR™1 compared to only 4–6 days after seeding in TeSR™2. Thus, cells grown in mTeSR™1 may need to be passaged approximately 1 day later than the same cells grown in TeSR™2.

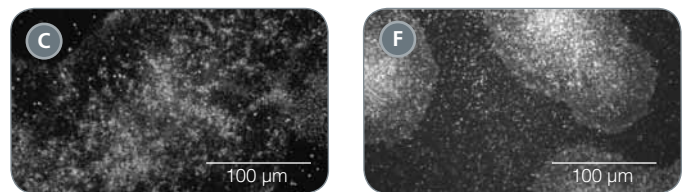
FIGURE 2. Morphology of representative hESC colonies in mTeSR™1 (A, B, C) and TeSR™2 (D, E, F).



Day 2: Colonies are small, transparent and not very densely packed with cells.



Day 4: Colonies rapidly increase in size and start to develop phase-bright centers when viewed under a phase contrast microscope. However, these colonies are not yet ready to be passaged. The colonies grown in TeSR™2 (E) shown here should be passaged within 24 hours.



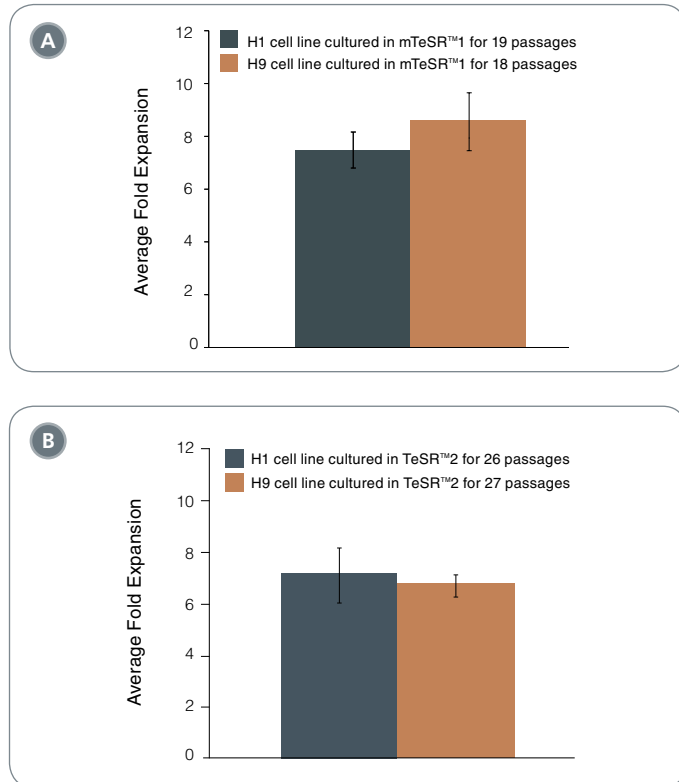
Day 5 or 6: At Day 6 colonies in mTeSR™1 (C) and at Day 5 colonies in TeSR™2 (F), are ready to passage. Colonies have begun to merge and have phase-bright centers that are densely packed with cells.

In either mTeSR™1 or TeSR™2 media, if colonies are passaged too early or too frequently, the cells may not attach well, yields will be decreased and cells may start to differentiate. If colonies are passaged too late, the culture will begin to show signs of differentiation. If the colonies are large with dense centers and are beginning to merge, cells should be passaged within 24 hours. Typically, expansion of cells is 7–10 fold per passage in mTeSR™1, compared to 6–8 fold per passage in TeSR™2 (Figure 3).

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FIGURE 3. Expansion of hESCs Cultured in mTeSR™1 (A) and TeSR™2 (B).



H1 and H9 hESC cultures show 7- to 10-fold expansion in mTeSR™1 and 6- to 8-fold expansion in TeSR™2 consistently across passages.

Enzymatic Dissociation

Because hESCs and hiPSCs cultured in mTeSR™1 are less sensitive to enzymatic dissociation than when cultured in TeSR™2, the time for incubation with Dispase (Catalog #07923; 1 mg/mL, specific activity 0.8–1.2 U/mL) should be increased from 3–4 minutes to approximately 7 minutes when cells are cultured in mTeSR™1. After incubation, the colony edges will appear slightly folded back but the colonies should remain attached to the plate. Please note that these time recommendations are based on dispase from STEMCELL Technologies. If dispase from another supplier is used, these times may need to be adjusted due to differences in enzyme activity.

Replating Efficiency

In most cases, the equivalent seeding density used for cells cultured in TeSR™2 can be applied to cultures in mTeSR™1. It is, however, recommended that the original TeSR™2 culture used for transitioning into mTeSR™1 medium is also maintained in parallel for 2–3 passages; this is to ensure that the chosen plating density during transitioning into mTeSR™1 is appropriate to establish viable and good quality undifferentiated colonies in mTeSR™1.

Transitioning Key Points

- **Growth Kinetics:** Cells ready for passage in 5–7 days in mTeSR™1 compared with 4–6 days in TeSR™2.
- **Enzymatic Dissociation:** Incubation typically needs to be longer for cells cultured in mTeSR™1 compared to TeSR™2 (3–4 minutes versus 7 minutes respectively using dispase from STEMCELL Technologies).
- **Replating Efficiency:** Equivalent in most cases, but a side-by-side comparison of mTeSR™1 and TeSR™2 cultured cells is recommended for 2–3 passages.

PRODUCT	QUANTITY	CATALOG #
mTeSR™1	500 mL	05850
	10 x 500 mL	05870
	25 x 500 mL	05875
	1L	05857

Support Products

PRODUCT	CATALOG #
AggreWell™400 plates	27845 / 27945
AggreWell™800 plates	27865 / 27965
AggreWell™ Medium	05893
Oct 3/4 Antibody, Clone 40	01550 / 01551
SSEA-1 Antibody, Clone MC-480	01552
SSEA-3 Antibody, Clone MC-631	01553
SSEA-4 Antibody, Clone 813-70	01554
STEMCircles™-LGNSO	05820
TRA-1-60 Antibody, Clone TRA-1-60	01555
TRA-1-81 Antibody, Clone TRA-1-81	01556
TRA-2-49 Antibody, Clone TRA-2-49/6E	01557
TRA-2-54 Antibody, Clone TRA-2-54/2J	01558
mFreSR™ Defined Cryopreservation Medium	05855 / 05854
Dispase (1 mg/mL)	07923

For a list of select mTeSR™1 publications, please visit:
www.stemcell.com/mTeSR1publications

References

- Ludwig TE et al., Nat Biotechnol 24:185-187, 2006
Ludwig TE et al., Nat Methods 3:637-646, 2006