

STANDARD OPERATING PROCEDURE

	INDUCED PLURIPOTENT STEM CELL CORE	iPSC PASSAGING PROTOCOL WITH VERSENE	
	THE DAVID and JANET POLAK FOUNDATION STEM CELL CORE LABORATORY	SOP NUMBER: SOP-iPSC-004	Version: B

1. PURPOSE

To describe the procedure for chemical passaging of iPSCs maintained on Matrigel using Versene

2. SUPPLIES

Complete mTeSR Medium (Basal medium + 5x Supplement) (StemCell Technologies, Cat # [05850](#))

Matrigel Coated TC dish (Prepared as described in SOP-iPSC-002)

Versene (Life Technologies, Cat # [15040-066](#))

5ml and 10ml sterile serological pipettes

3. PROCEDURE

NOTE: You must have a prepared Matrigel coated plate before starting this protocol. If you are using a Matrigel coated plate that has been stored at 4°C, **the plate must be allowed to equilibrate to room temperature for 1 hour prior to starting.**

- 3.1 Prior to passaging your cells, check colonies in a microscope and using a cleaning tool remove any areas of differentiation from the culture.
- 3.2 Aspirate Matrigel from the prepared Matrigel coated tissue culture plate and add 2mls of complete mTeSR media to each well (for a total of 2.5mls per well after cells have been added).
- 3.3 Aspirate spent media.
- 3.4 Rinse the wells with one volume of Versene and aspirate
 - 3.4.1 Use 1ml for a single well of 6-well plate
 - 3.4.2 Use 1.5ml for a single 60mm dish
 - 3.4.3 Use 0.5ml for a single well of a 12-well dish
- 3.5 Add a volume of Versene to each well.
- 3.6 Incubate at 37°C for 4-5 minutes.

Optional: Check cells under microscope after 4-5 minutes to check for the breaking up of colonies.



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3.7 Aspirate Versene and gently rinse the wells with a single volume of mTeSR.

NOTE: Typically, the cells should **NOT** lift from the plate at this point. You will lose a minimal number of cells. **If too many cells have lifted off the plate**, add additional media to the well and collect the cells into a sterile 15ml conical. Centrifuge the conical for 1 minute at 1000rpm. Proceed to step 3.8.

3.8 Add an appropriate volume of mTeSR and pipette up and down to dislodge the cells.

NOTE: If you have collected and centrifuged your cells, you will use this step to break up your cell pellet.

3.9 Pass cells at desired density into a new Matrigel coated TC dish.

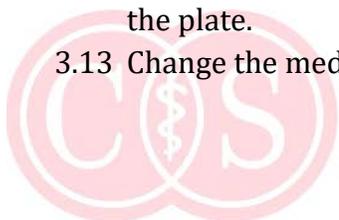
EXAMPLE: Add 3mls of mTeSR at step 3.8, then distribute 0.5ml of cell suspension to each well for a 1:6 split.

3.10 Place the plate in the 37°C incubator with 5% CO₂ and gently rock the plate back and forth and side-to-side to ensure even distribution of the colonies throughout the well.

3.11 Do not move the plate for 24 hours.

3.12 After 24 hours, view the plate in the microscope to confirm that the colonies have attached to the plate.

3.13 Change the media every day until ready to be used or passaged again.



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4. Troubleshooting

PROBLEM	POSSIBLE CAUSE	SOLUTION
Colonies are still whole after 5-minute incubation	Cells may need a longer incubation period	Place the plate back into the incubator and check for colony break up every 3 minutes
Colonies will not dislodge after 5-minute incubation	Cells may need a longer incubation period	Place the plate back into the incubator and check for colony break up every 3 minutes
Colonies have all attached to the center of the plate	Colonies did not evenly spread across the plate	<p>Make sure to perform step 3.10. Perform this step in the incubator so after the plate is rocked back and forth it won't be disturbed</p> <p>Place plates in the back of the incubator to ensure they are not bumped after passing</p> <p>Open and close the incubator door gently to prevent any dislodging</p>
Colonies did not attach to the plate after passaging	<p>Matrigel plates were old or died out</p> <p>Cell line may have difficulties attaching</p>	<p>Ensure that you are using a Matrigel plate that is no more than 1 week old and that every well is properly coated</p> <p>Cell line may require a double concentration of Matrigel. Coat a new plate.</p>