

Pluripotent Stem Cell Culture EDTA passaging

Required Reagents:

Reagent	Vendor	Catalog number
Nunc delta treated plates	Fisher Scientific	various
Matrigel (Growth Factor Reduced)	Corning	354230
Vitronectin (VTN-N)	Thermo Fisher / Life Technologies	A14700
PBS (-/-)	Thermo Fisher / Life Technologies	20012027
0.5M EDTA	Various	
Essential 8 Medium	Thermo Fisher / Life Technologies	A1517001
DMEM-F12	Thermo Fisher / Life Technologies	11330032

Required Equipment / Consumables:

- Biosafety Cabinet
- CO₂, O₂, humidified, water jacketed incubator (Thermo Fisher model 3130 or the like)
- 0.2 µm disposable filter units (Millipore or the like)
- Sterile, glass, borosilicate pipets (Fisherbrand)
- Sterile pasteur pipets

Prepare EDTA solution:

- Prepare a 0.5mM EDTA solution by diluting 0.5M EDTA 1:1000 in PBS (-/-)

Prepare ECM Plates:

- Aliquot Matrigel or VTN-N according to manufacturer's instructions. Freeze aliquots at -20C
 - The ratio of Matrigel we use is .0083 mg /cm², or .5mg per 6 well plate or 10cm² dish
- To prepare tissue culture plates, take 1 aliquot of matrigel or VTN-N and quickly thaw in cold DMEM-F12 and plate in the appropriate sized cell culture vessel.
 - Example: we use 1mL of DMEM-F12 per well of a 6 well plate or 5mL per 10cm² dish.
- Allow ECM to attach to plate by incubating at 37C for 30 minutes, room temperature for 1 hour or overnight at 4C. Store ECM coated plates at 4C taking care not to allow the media to evaporate off of the dishes.

Passaging

1. Remove plate to be passaged from incubator and place it in the biosafety cabinet.
2. Remove the spent medium from the wells to be passaged using Pasteur pipette.
3. Rinse each well to be passaged with 1ml room temperature PBS.
4. Add 1ml room temperature 0.5mM EDTA to each well to be passaged.
5. Incubate for 2-7 minutes at room temperature. ****Do not incubate until cells detach****, they should be loosely adherent to the culture plate and easily able to slough off the plate with gentle washing. If cells detach, collect and centrifuge the cells, resuspend in an appropriate volume for the proper passage ratio, and add the resuspended cells to new Matrigel or VTN-N coated plates.
6. During the EDTA treatment, remove basal media from Matrigel or VTN-N plates and add 2mL of Essential 8 to each well of a 6 well plate. Scale volumes accordingly if your culture vessel is different.
7. Once incubation is complete, aspirate the EDTA and collect cells to be passaged using fresh E8 media using a 5mL pipet.
8. Place culture vessel into the incubator and gently shake the plate back and forth and front to back to evenly distribute the cells—avoid circular motions to prevent concentrating cells in the middle of the well.
9. Cells will attach within 1-2 hours however allow them to incubate overnight for optimal cell health
10. Continue to feed cells until needed for experiments, passaging or freezing
11. In our hands, robust pluripotent stem cells (such as H1) can be passaged 1:12 every 3-5 days. It is important to not let cells become overconfluent for optimal cell health.