[Protocol 1.02.0] Chemical passaging of iPSC using EDTA

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Protocol 1.02.0 Chemical passaging of iPSC using EDTA
Version: 1.0 (20.12.2021)

Media and Reagents:
optimised Medium for species (*refer to protocol 1.07, 1.08 or 1.09*)
UltraPureTM 0.5 M EDTA (Thermo Fisher,15575-020)
PBS w/o Ca2+, Mg2+ (Life Technologies, 14190250)

Materials and Equipment:
Coated culture vessels(*refer to protocol 1.05 or 1.06*)
Aspiration Pump
Cell scraper
5 mL, 10 mL pipettes

1. Introduction and Purpose
This protocol describes the procedure for using EDTA to chemically passage induced pluripotent stem cells (iPSC) cultured in Geltrex or Matrigel coated 6 well plates. The passaging with EDTA results in cluster splitting, meaning the cells should stay as clusters **not** as single cells in order to maintain the cell viability. Therefore, this passaging method is mainly aimed for maintaining iPCSs.

2. Chemical passaging with 0,5 mM EDTA
Depending on the growth characteristics of specific cell line, cells must be passaged every 3-5 days in order to maintain log phase of growth and avoid induction of differentiation. Differentiation can easily be initiated, if cell density becomes too high. Established cultures are usually splitted to 1:10 and for the backup to 1:20. Please have always a backup well for cases such as differentiation, contamination, etc.

1. If cells will be passaged to 6 well, prepare the previously coated culture vessels by aspirating residual coating solution and adding 1.5 ml of medium.
2. Place the vessel with media into the incubator (pre-warming to 37°C) until cell seeding (*For seeding, please refer to the 8. step in this protocol*).
3. Use a microscope to visually identify regions of differentiation and remove these regions with a scraping tool *See protocol 1.1 Chapter 3.*
4. Aspirate medium from the culture and rinse once with 1 ml of PBS w/o Ca2+, Mg2+.
Note: For preparation of 0.5 mM EDTA, refer to protocol 1.2.1!
5. To detach cells add 1 ml of 0.5 mM EDTA to the culture vessel.
on Geltrex incubate at 37°C for 3 - 5 minutes
on Matrigel incubate at room temperature (~21°C) for 5 - 7 minutes
Note: When cells will start to separate, round up and the colonies will appear to have holes in them, they are ready to be removed from the vessel. Care should be taken not to incubate until the colonies detach from the plate *See reference images in appendix!*
6. Aspirate EDTA gently and add 3 ml of culture medium to one well.
7. Firmly tap the side of the plate for approximately 5 - 10 seconds or use a scraper to gently dislodge the cells from the vessel. Pipet cell suspension one or two times up and down using a 5 ml pipette. Do not pipet up and down more than 2 times to avoid breaking the cell clumps into single cells.
Note: Never use microliter pipette tips during these steps, resulting sheer forces are very insensitive and will break the cell clusters down to single cells.
8. Seed the cells at an appropriate cell density (see below) by transferring the required volume of cell suspension to the prepared plate/well pre-filled with medium.

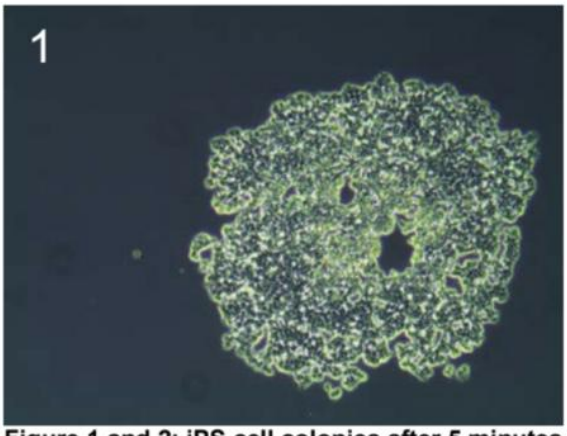
Split ratio	Transfer volume to new 6 well [µl]
1:6	500
1:10	300
1:12	250
1:20	150

9. Rock the plate forth and back to guarantee an equal distribution of cell cluster throughout the whole well. Place the plate into the incubator (37°C, 5% CO2) or hypoxic conditions (5% O2).

Relevant applicable documents:
protocol 1.02.1 Preparation of of 0.5 mM EDTA
protocol 1.05 Geltrex coating of culture vessels
protocol 1.06 Matrigel coating of culture vessels
protocol 1.07 Preparation of E8 medium
protocol 1.08 Preparation of mTeSR1 medium
protocol 1.09 Preparation of stemMACS/UPPS medium

Screenshot_2021-12-20_at_11.03.50.png

Images of EDTA treated colonies



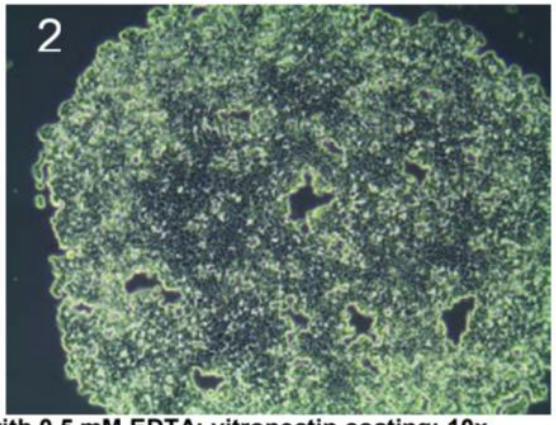


Figure 1 and 2: iPS cell colonies after 5 minutes incubation with 0.5 mM EDTA; vitronectin coating; 10x

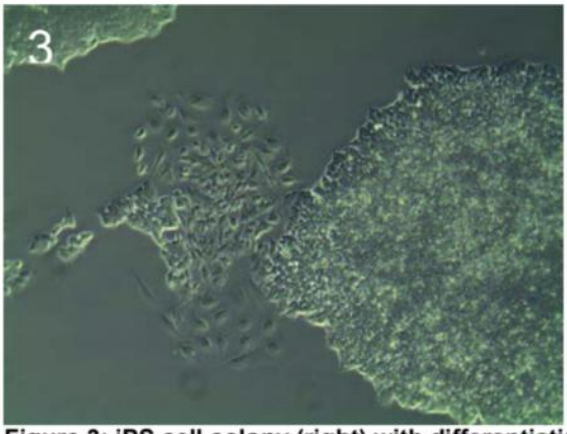


Figure 3: iPS cell colony (right) with differentiating cells (Middle/ left) vitronectin coating; 10x

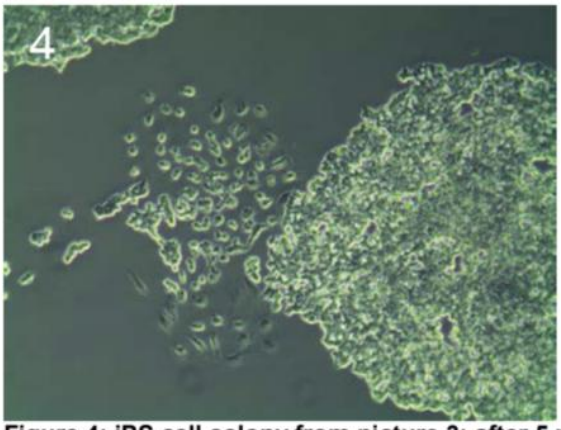


Figure 4: iPS cell colony from picture 3; after 5 minutes incubation with 0.5 mM EDTA, vitronectin coating; 10x