**EDTA clump passaging of iPSCs**

Materials

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| **Item** | **Cat#** | **Manufacturer** |
| UltraPure™ 0.5M EDTA, pH 8.0 | 15575020 | Invitrogen |
| DPBS, no calcium, no magnesium | 14190235 | Gibco |
| Plate/flask coated with preferred adherence matrix (e.g. Matrigel or Geltrex) | | |
| Warm iPSC media (e.g. mTeSR or E8) | | |

Protocol

1. Prepare 0.5 mM EDTA solution in DPBS (1:1000 dilution from 0.5M EDTA stock, i.e. 50 µl in 50 ml DPBS).

Store solution at RT.

1. Aspirate medium from the well(s) you are splitting and wash well(s) once in DPBS.
2. Add 1 ml 0.5 mM EDTA solution per well of a 6-well plate. Scale up or down as required for other plate/flask formats.
3. Leave the EDTA solution on and incubate at 37°C for 5 mins. During these 5 mins, label your new plate/flask and add warm media to it.
4. Optional: After 5 mins, check your plate under a brightfield microscope to see if the cells are ready to detach (increased contrast around the cell perimeter).
5. Carefully aspirate the EDTA solution (this will not aspirate living cells).
6. Collect the cells into a P1000 pipette tip by “shooting” medium once over the well. If you do not collect most cells in one go, you can go over it once more, but ideally no more than twice (more will break up the iPSC clusters too much, and healthy iPSCs normally readily detach). A good iPSC split ratio for one healthy and confluent well is generally 1:6. If the yield is lower, plate your collected iPSC clusters into fewer wells.

NB: iPSCs split 1:6 with clump passaging (EDTA/ReLeSR) do not generally need to be fed until 48h after splitting (then every day feeding commences). Addition of ROCK inhibitor is not advised when performing clump passaging.