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Human induced pluripotent stem cell culture

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gurvir.virdi¹

¹UCL Institute of Neurology



gurvir.virdi

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Protocol status: Working

We use this protocol and it's working

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Abstract

Cell culture for the maintenance of hiPSCs.



- 1 Coat 6 well plates with Geltrex LDEV-Free Reduced Growth Factor Basement Membrane Matrix (Thermo Fisher Scientific), 1:100 dilution for 1 hour before cell passaging.
- 2 When hiPSCs are 80-90% confluent, they are passaged:
 - 2.1 Old media is aspirated and replaced with 1ml of cell-culture PBS (Gibco, Thermo Fisher Scientific)
 - 2.2 PBS is aspirated and replaced with 0.5 mM EDTA (Thermo Fisher Scientific) made up in PBS.
 - 2.3 hiPSC are incubated in EDTA for  00:05:00 at  37 °C .
 - 2.4 After 5 minutes, the EDTA is aspirated and cells are dissociated with media (E8 or mTesR 1).
 - 2.5 hiPSCs are then placed in new 6 well plates at the desired dilution.
- 3 All hiPSCs are fed daily with fresh media. All cells were maintained at 37°C and 5% carbon dioxide.

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