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Harvesting protocol for H1 and H1 Endoderm cells

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1. On the day of harvesting, cells were washed with PBS and dissociated in to single cells using TripLE Express reagent for 3-5min at 37^{o}C.
2. Cells were collected by adding PBS and spun down at 1000rpm for 5min at RT.
3. Cell pellet was resuspended in 10ml of cold PBS, washed at 1000rpm for 5min at 4^{o}C and discorded the supernatant.
4. Next, pellet was resuspended in 10ml of ice cold PBS for cell counting. Cell number was estimated using cell counter and performed second PBS wash at 1000rpm for 5min at 4^{o}C.
5. After second PBS wash at 1000rpm for 5min at 4^{o}C, the pellet was resuspended in about 10 x 10^{6} cells per ml of cold PBS and transferred in to fresh eppendorf tubes as required.
6. Cells were spun again at 1000rpm for 5min at 4^{o}C and discorded the supernatant.
7. Finally, cell pellet was flash frozen in liquid N2 for 30sec and then stored in -80^{o}C for the shipment.
8. For cryopreservation, cell pellet was resuspended in cold 10% DMSO (in PBS) by adding drop-wise on the walls of the cryo tubes, and performed slow cooling using Mr Frosty container in -80^{o}C for the shipment.