**Table 1: TROUBLESHOOTING**

|  |  |  |  |
| --- | --- | --- | --- |
| **STEP** | **ISSUE** | **POSSIBLE REASON** | **POTENTIAL SOLUTION** |
| 1 | Slow growth of fibroblasts during proliferation | Passage number too high | Allow more time for growth, giving more regular media changes. In future revive lower passage cells |
| 1 | Cells not detaching well with trypsin | Cell line variation/age/time elapsed | Incubate the cells in trypsin at 37°C for a few minutes, tapping regularly to check detachment progress. |
| 2 | Low transfection efficiency | Refer to Troubleshooting documents on the manufacturer’s website. | Refer to Troubleshooting documents on the manufacturer’s website. |
| 2 | Low survival of cells following transfection | Refer to Troubleshooting documents on the manufacturer’s website. | Refer to Troubleshooting documents on the manufacturer’s website. |
| 3 | Neurospheres not forming | Cell line variation/days in culture | Neurosphere formation is not a definitive sign of neural reprogramming. Check for iNP marker expression regardless. |
| 3 | Cells not thriving | Midkine present too long | Check media components are correct |
| 3 | Cells peeling or forming dense dark clumps or spheres | Cell density may be too high | Replate the cells at ~31,250 cells/cm2. More than weekly may occasionally be necessary depending on the cell line. |
| 4 | Low RNA yield for qPCR | Inefficient iNP formation | Allow for more loss of cells when calculating the number of fibroblasts to transfect |
| 5 | iNPs peel off during immunocytochemistry | Cell density too high | Lower density is better for retention of cells and imaging for immunocytochemistry. Ensure the surface is coated and consider using glass coverslips in 24-well plates. |
| 6 | Cell detachment during differentiation | Cell density too high/cells unhealthy | Some detachment and/or death is normal at this stage, however this can be reduced by extremely gentle, regular media changes. Using coated, flat surfaces is critical. |
| 6 | iNPs do not differentiate | Timing of iNP differentiation wasn’t optimal | Try plating for differentiation later, or earlier, based on iNP marker expression at various timepoints. |
| 6 | Fibroblasts crowding differentiating cells | Timing of iNP differentiation wasn’t optimal | Allow longer for the iNPs to form (and remaining fibroblasts to be lost) in culture before plating for differentiation |
| 7 | Neurons not expressing mature markers (or not performing functionally via electrophysiological measures) | Neurons weren’t given long enough to mature | Keep neurons in differentiation culture conditions for longer |