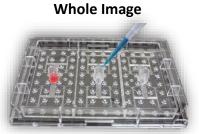
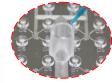
Full Protocol of 1CellPlate for Single-Cell Cloning

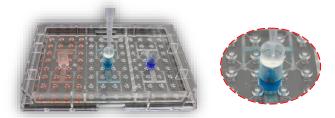






Step 1: Cell Suspension Preparation

- 1. Harvest adherent cells and make them into cell suspension.
- 2. Transfer 2 µl of cell suspension to a Petri Dish.
- 3. Identify the cell suspension and roughly estimate cell numbers under the microscope.
- 4. The ideal number of cells is 25-35. If too much/little, dilute/enrich it to match the number.
- 5. Add 200 µL of culture medium to the cell suspension and pipette cells to be fully suspended.
- 6. Transfer 200 μl of cell suspension into one Inlet Port of 1CellPlate.



Step 2: Single-Cell Isolation

- 1. Put a pipette tip containing an Inlet Adaptor on the top of Inlet Port.
- 2. Press pipette plunger down (200 μ L +P) to aliquot cell suspension into the 32 Outlet Wells.
- 3. Add 200 μL of culture medium into the Inlet Port to prevent back flow.



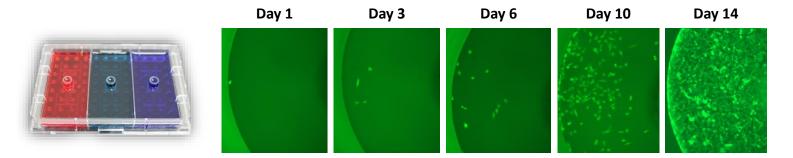
Step 3: Single-Cell Identification

- 1. Put the 1CellPlate under microscope with 10x objective (bright field or fluorescence).
- 2. Identify and note all Outlet Wells containing only single cells.



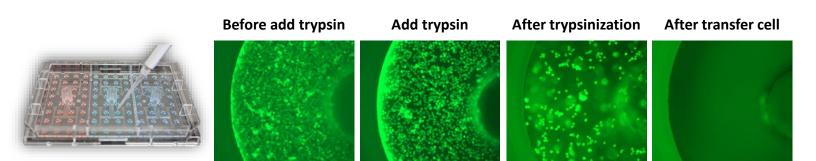
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Full Protocol of 1CellPlate for Single-Cell Cloning



Step 4: Single-Cell Cloning

- 1. Add 10 mL of cell culture medium into each large Chamber.
- 2. Culture the isolated single cells for ~2 weeks to generate clonal cells.



Step 5: Clonal Cells Harvesting

- 1. Remove cell culture medium from the Chamber.
- 2. Wash residual medium 2-3 times by PBS and then remove PBS from the Chamber.
- 3. Carefully remove PBS from the Outlet Wells containing desired clonal cells.
- 4. Add about 2 μl of trypsin into the Outlet Wells containing desired clonal cells.
- 5. Put the 1CellPlate into the cell culture hood for 3-5 minutes to dissociate adherent cells from the bottom.
- 6. Use pipette to harvest the clonal cells from the Outlet Wells of 1CellPlate.



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