iBiochips

Protocol for 1CellAssay® 3D Culture Kit

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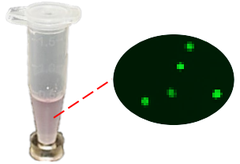
Prepare Cells & Hydrogel

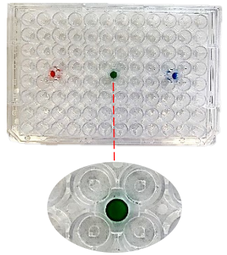
Warm the hydrogel at room temperature or to 37°C. Prepare 120 µL of cell suspension with 650-900 cells/mL. A pre-prepared conditioned cell culture medium is typically recommended to use in promoting single-cell growth.

Mix Cells & Hydrogel



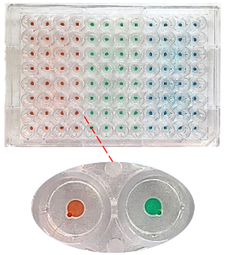
Fully suspend the cells and pipette up 120 µL of cell suspension (containing 78-108 cells). Mix the 120 µL of cell suspension with 240 µL of hydrogel to generate a 360 µL of the cell-hydrogel mixture.

Add Cell-Hydrogel Mixture



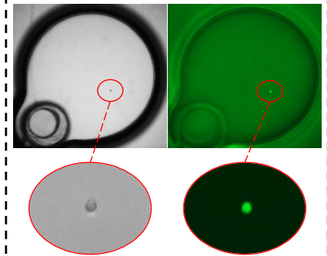
Find the three Inlet Ports on 1CellPlate-96well. After mixing with a pipette (Do not generate air bubbles), rapidly transfer 110 µL of the cell-hydrogel mixture (containing 26-36 cells) into each Inlet Port.

Isolate Single Cells



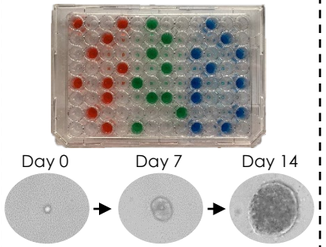
Attach the provided pipette tip with Inlet Adaptor to a pipette and set it to 110 µL. Put the Inlet Adaptor on top of an Inlet Port. Press the pipette to its first stop and hold the pressure for ~15 s to aliquot cell-hydrogel mixture.

Identify Single Cells



Check all the wells containing single cells with a microscope. Bright-field or fluorescence with a 10x objective is typically recommended. Write down well labels with target single cells. The total yield should be ~30.

3D Culture Single Cells



After isolation, wait ~15 min at room temperature and add 100-200 µL of the (conditioned) culture medium into each well containing a single cell. Culture to generate a 3D cloning, such as a spheroid or an organoid.

Applications

- Single-Cell Isolation from Cell Lines and Primary Cells
- Single-Cell 3D Clonal Culture
- Generation of Clonal Spheroids & Organoids for Drug Screening

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