PROTOCOL

CELL PROLIFERATION ASSAY (USING CCK-8 ASSAY)

RECOMMENDED MATERIALS AND REAGENTS

- Cells cultured with VitroGel system
- Cell Counting Kit- 8 (CCK-8) assay
- Cells
- VitroGel hydrogel
- Cell culture medium
- Micropipette
- Plate reader

PROTOCOL:

MAKING A STANDARD CURVE

(Using a 96 well-plate, 50 µL gel/well with 50 µL cover medium as an example)

1. Prepare the cell suspension in the culture medium with a gradient of cell numbers. (e.g., cell # $1x10^5$ to $5x10^6$ cells/mL).
2. Mix the VitroGel hydrogel with the cell suspension according to the VitroGel user handbook.
3. Add 50 µL hydrogel-cell mixture to each well of a 96 well-plate.
   Note: The final gradient of cell numbers in each well should be from 1,000 cells/well to 25,000 cells/well with 5-7 gradient points in between and 3-5 repeats at each point).
4. Wait 10-20 minutes for the hydrogel to stabilize, then add 50 µL cell culture medium to cover the hydrogel.
5. Incubate at 37 °C for about 2-4 hours.
6. Add 10 µL of CCK-8 to the cover medium on top of hydrogel. Gently swirl the plate to make sure CCK-8 is evenly distributed.
7. Incubate the plate in the dark (keeping away from light) at 37 °C for about 2 hours.
8. Before reading the plate, swirl the plate gently to ensure homogeneous distribution of color.
9. Measure the absorbance at 450 nm using a microplate reader.
10. Make a standard curve using the cell numbers as the X-axis and the O.D. value as the Y-axis.

Note: A prerequisite for using this standard curve is that the cell culture conditions are the same.

CELL PROLIFERATION ASSAY

1. Culture cells with VitroGel in a 96-well plate (50 µL hydrogel and 50 µL cover medium for each well).
2. Add 10 µL of CCK-8 solution to each well of the plate. Gently swirl the plate to make sure CCK-8 is evenly distributed.
   Note: Be careful not to introduce bubbles to the wells as they interfere with the O.D. reading.
   Optional: Remove 50 µL cover medium and add fresh 50 µL cover medium before adding the CCK-8 solution.

CELL PROLIFERATION ASSAY (Continued)

3. Incubate the plate in the dark (keeping away from light) at 37 °C for about 2 hours.
4. Before reading the plate, swirl the plate gently to ensure homogeneous distribution of color.
5. Measure the absorbance at 450 nm using a microplate reader.
6. Record the O.D. value and use the standard curve to convert the O.D. value to cell number.
7. Repeat steps 2-6 at different time points (e.g. every 24-48 hours).