

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 μ L gel/well with 50 μ L cover medium as an example)

- 1. Prepare the cell suspension in the culture medium with a gradient of cell numbers. (e.g., cell $\# 1 \times 10^5$ to 5×10^6 cells/mL).
- 2. Mix the VitroGel hydrogel with the cell suspension according to the VitroGel user handbook.
- 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 μ L 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.

<u>Note</u>: Be careful not to introduce bubbles to the wells as they interfere with the O.D. reading. <u>Optional</u>: Remove 50 μ L cover medium and add fresh 50 μ L 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).



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