PROTOCOL

CELL LYSIS FOR DOWNSTREAM ANALYSIS

RECOMMENDED MATERIALS AND REAGENTS

- Cells cultured with VitroGel system
- VitroGel Cell Recovery Solution (Cat# MS03-100)
- DPBS (no calcium, no magnesium)
- Cell lysis buffer
- Conical tubes (15 mL or 50 mL) or Microcentrifuge tube
- Serological pipettes or Micropipette; Low retention pipette tips
- Lab probe sonicator
- 37 °C water bath or dry bath
- Centrifuge

PROTOCOLS: (using 96 well-plate, 50 µL gel/well as an example)

Protocol 1: Cell lysis with hydrogel together

1. Remove the medium covering the top of the hydrogel and wash the hydrogel three times with DPBS.
2. Add 100 µL iced cold lysis buffer to hydrogel and mix the gel and buffer by pipetting up and down.
3. Transfer the mixture to a microcentrifuge tube and incubate on ice for 15 minutes.
4. Sonicate the mixture by using a lab probe sonicator: sonicate 2-5 seconds and rest for one minute on ice, repeat three times.
5. Incubate the mixture an additional 15 minutes (optional).
6. Centrifuge at 13,000 x *g* for 5 minutes at 4 °C.

Protocol 2: Harvest cells from hydrogel before cell lysis

1. Warm the VitroGel Cell Recovery Solution to 37 °C.
2. Take the cells out of the incubator and remove the medium covering the top of the hydrogel. Wash the hydrogel two times with DPBS.
3. Add 100 µL warm VitroGel Cell Recovery Solution and mix the gel and solution by pipetting up and down 5-10 times.
4. Transfer the mixture to a microcentrifuge tube containing the 0.5-1 mL warm VitroGel Cell Recovery Solution.
5. Gently pipette the mixture up and down 5-10 times and incubate at 37 °C for 2-3 min. Repeat 3 times.
6. Centrifuge at 100 x *g* for 3-5 minutes at room temperature to collect the cell pellet (optimize the speed and time of centrifuge according to different cell types).

   Optional: If there is still some hydrogel on top of the cell pellet, resuspend the cell with 1 mL warm cell recovery solution and repeat steps 5 and 6 one more time.

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7. Remove the supernatant and add 100 µL iced cold lysis buffer to the cell pellet.
8. Resuspend the cells in lysis buffer and incubate on ice for 15 minutes.
9. Sonicate the mixture by using a lab probe sonicator: sonicate 2-5 seconds and rest for one minutes on ice, repeat three times.
10. Incubate the mixture an additional 15 minutes (optional).
11. Centrifuge at 13,000 x *g* for 5 minutes at 4 ºC.