# OriCell PlatE Cell Line Instructions for Use

Catalog No. HEKPE-30001

## Materials Required (not supplied)

1. Trypsin-EDTA (Cat. No. TEDTA-10001-100）
2. Phosphate-Buffered Saline (1×PBS) (Cat. No. PBS-10001-500)
3. PlatE Cell Growth Medium (Cat. No. HEKPE-90011)

## Thawing of PlatE Cell

1. Prepare 37°C water bath and pre-warm PlatE Cell Growth Medium to 37°C.
2. Add 9mL of PlatE Cell Growth Medium to a 15mL conical tube.
3. Remove the cryovial of PlatE Cell from liquid nitrogen. Quickly thaw the vial in 37°C water bath until the last crystal piece disappears, and finish the thawing procedure within 3 minutes. Be careful not to submerge the entire vial. Maximum cell viability is dependent on the rapid and complete thawing of frozen cells.

**Note: Thawing the cells for longer than 3 minutes results in less than optimal results.**

4. As soon as the cells are completely thawed, disinfect the outside of the vial with 70% ethanol.
5. In a laminar flow hood, use pipette to transfer the cells to the conical tube containing PlatE Cell Growth Medium. Be careful not to introduce any bubbles during the transfer process.
6. Rinse the vial with 1mL of medium to reduce the loss of cell and then transfer this 1mL of cell suspension to the conical tube.
7. Gently mix the cell suspension by slowly pipeting up and down. Be careful not to introduce any bubbles.
8. Centrifuge the cell suspension at 250 g for 5 minutes.
9. Carefully aspirate as much of the supernatant as possible and add 2-3mL of fresh PlatE Cell Growth Medium (pre-warmed to 37°C).
10. Gently re-suspend the cells in PlatE Cell Growth Medium.
11. Plate the cells into a T25 flask and add sufficient PlatE Cell Growth Medium. Gently rock the culture flask to evenly distribute the cells.
12. Incubate at 37°C in a 5% $CO_2$ humidified incubator.
13. The next day, change the medium with fresh PlatE Cell Growth Medium (pre-warmed to 37°C).

14. Change the growth medium every three days thereafter.
15. When the cells are approximately 80 to 90% confluence, they can be dissociated with Trypsin-EDTA and passaged.

## Changing Medium

1. Warm an appropriate amount of medium to 37°C in a sterile container. Remove the medium and replace it with the warmed, fresh medium and return the flask to the incubator.
2. Avoid repeated warming and cooling of the medium. If the entire contents are not needed for a single procedure, transfer only the required volume to a sterile secondary container.

## Subculturing

1. Pre-warm the PlatE Cell Growth Medium, 1×PBS, Trypsin-EDTA solution to 37°C.
2. Carefully aspirate spent medium from the 80 to 90% confluent monolayer of PlatE Cell.
3. Add 1×PBS (6mL for T75 flask, 3mL for T25 flask). Be careful not to disturb the monolayer. Rinse the monolayer by gently rocking the flask back and forth.
4. Aspirate 1×PBS and discard.
5. Repeat the step 3-4 two or three times.
6. Add Trypsin-EDTA solution (1.5mL for T75 flask, 0.5mL for T25 flask). Gently rock the flask back and forth to ensure that the entire monolayer is covered with the Trypsin-EDTA solution. Allow the trypsinization to continue until the majority of the cells (approximately 80%) are rounded up. At this point, gently tap the side of the flask to release the majority of cells from the culture surface.

**Note: Avoid leaving cells exposed to the trypsin longer than necessary. Care should also be taken that the cells not be forced to detach prematurely, as this may result in clumping.**

7. After the cells are visibly detached, immediately add PlatE Cell Growth Medium (pre-warmed to 37°C) (6mL for T75 flask, 3mL for T25 flask) to neutralize the trypsinization.
8. Gently pipet the medium over the cells to dislodge and re-suspend the cells. Repeat 5-6 times until all the cells are dissociated from the flask and evenly dispersed into a single cell suspension.

**Note: Care should be taken to avoid introducing bubble during pipeting.**

9. Transfer the dissociated cells into a 15mL conical tube.
10. Centrifuge at 250 g for 5 minutes to pellet the cells.
11. Carefully aspirate as much of the supernatant as possible.
12. Add 2mL of PlatE Cell Growth Medium to the conical tube and re-suspend the cells thoroughly but gently.

13. Plate the cells into appropriate flasks. PlatE Cell can be split at 1:3-10 or other appropriate ratio.

14. Add sufficient medium.

15. Incubate the cells at 37°C in a 5% $CO_2$ humidified incubator.

## Hints

### Time to Change Medium

Although the cells do not reach 80 to 90% confluence, if the medium becomes acidic (the pH indicator in culture medium appears yellow), it is recommended that the medium be changed. In general, change the growth medium every three days.

### Time to Subculture

When PlatE Cell reach 80 to 90% confluence, it is recommended that the cells be subcultured. Don't let PlatE Cell overgrow, or it will result in contact inhibition.