RSAR001 user's manual

Culture Media

- RPMI1640 medium with 15% heat-inactivated FBS

Reagents

- Sterilized D-PBS
- PBS-EDTA
- Trypsin-EDTA*
- Trypsin neutralizing solution*
- HEPES buffered saline solution*

  *Recommend to use "ReagentPack™ Subculture Reagents" (Lonza, CC-5034)
- Cryopreservation medium

Handling procedure for frozen cells

1. Add 15 ml of medium into 50-ml sterile tube in a biological safety cabinet.
2. Thaw the cells by gentle agitation in a 37 °C water bath for 1 minute.
3. Take the vial out of the water bath, wipe with 70% ethanol, then into the biological safety cabinet.
4. Transfer the vial contents into a sterile tube containing 15 ml of medium.
5. Mix the cells and medium gently.
6. Transfer the cell suspension to a T75 flask.
7. Incubate the culture at 37 °C, 5% $CO_2$.
8. Next day, remove all culture medium from the T75 flask, and add 15 ml of fresh medium.

Subculturing procedure

1. Remove culture medium from the T75 flask.
2. Rinse twice the cell layer with 10 ml of PBS-EDTA.
3. Rinse the cell layer with 6 ml of HEPES buffered saline solution.
4. Add 5 ml of Trypsin-EDTA to flask and incubate at room temperature for 5 minutes.
5. Add 10 ml of Trypsin neutralizing solution and gently pipette the cell suspension.
6. Transfer the culture suspension to a sterile centrifuge tube and centrifuge at 200 x g for 3 minutes at room temperature.
7. Discard the supernatant.
8. Resuspend the cell pellet in 10 ml of D-PBS.
9. Centrifuge at 200 x g for 3 minutes at room temperature.

10. During centrifugation, add 14 ml of medium into a T75 flask.
11. Discard the supernatant.
12. Resuspend the cell pellet in 3 ml of fresh medium and add 1 ml of the cell suspension into the T75 flask containing 14 ml of medium (1/3 split).
13. Incubate culture at 37 °C.

Cryopreservation procedure

1. Follow the step 1 to 9 of “Subculturing procedure”.
2. Discard the supernatant.
3. Resuspend the cell pellet in 3 ml of cryopreservation medium.
4. Add 1 ml each of cell suspension into three cryopreservation vials.
5. Place the vial in a cryopreservation container and freeze in a -80 °C freezer.
6. Store the cryopreserved vial in vaper phase of liquid nitrogen.