FAQs

1. **What are the precipitates in serum? Will they affect cell culture?**

   The following types of precipitates can be found in serum for cell culture: (1) Fibrin is a commonly visible precipitate in serum with a diameter of 1-2 mm. Serum is kept cold throughout all collection and manufacturing processes, during which fibrinogen is in a dissolved state. However, after filtration processes, serum fibrinogen tends to aggregate and precipitate. (2) Calcium phosphate, another common precipitate which leads to turbidity. This phenomenon is particularly noticeable when the serum is incubated at 37°C. Under an inverted microscope, calcium phosphate precipitates (seen as black dots) can be observed in irregular Brownian motion, which sometimes being regarded as microbial contamination by mistake. (3) Other components in serum, such as cholesterol, fatty acid esters and some proteins, can also form precipitates.

   These precipitates have no influence on cultured cells, thus don't have to be removed by additional filtration processes. If the presence of flocculent material or turbidity is a big concern, it can be removed by performing a filtration process after being added to the cell culture medium.

2. **How to avoid the occurrence of precipitates in serum?**

   The serum should be thawed in a gradual way: The frozen serum (usually at -20°C) has to be thawed mildly at 4°C. Please avoid directly thawing at room temperature. During the thawing process, shaking the bottle evenly and gently at intervals. Following conditions may lead to the increase of precipitates: heat inactivation, incubate at 37°C, repeated freeze-thaw cycles, γ-ray irradiation, long-term storage at 2-8°C, and placing at room temperature for prolonged periods of time.

   [Note]: To avoid contamination, the serum should be subpackaged and stored at -20°C.

3. **What is the role of L-glutamine in cell culture? Is it unstable in medium?**

   After removal of the amino group, L-glutamine can be used as a source of energy for cultured cells, involved in protein synthesis and nucleic acid metabolism. Meanwhile, the degradation of L-glutamine leads to the production of ammonia which is toxic to the cells. Since L-glutamine degrades over time in the medium, it is recommended to supplement additional L-glutamine into the medium in time after a period of culture.

4. **Is it necessary to add antibiotics in the medium?**

   In general, 1% penicillin-streptomycin is added to the culture medium to prevent contamination of bacteria. For special requirements, no antibiotics or other types of antibiotics can be added.

5. **How to avoid the contamination of cells?**

   The contaminants of cells can be bacteria, yeasts, molds, viruses and mycoplasma. The main causes of contamination include improper operation of aseptic technique, poor experimental

environment, contaminated reagents and supplies for cell culture, contaminated cells, *etc.* Therefore, the best ways to prevent cell contamination are related to strict aseptic technique, clean operating environment and well-qualified cell sources.

6. **How to deal with contaminated cells?**
   When cell contamination occurs, the contaminated cells should be directly sterilized and discarded to avoid the contamination of other cell lines.

7. **Can the abnormal state of mycoplasma contaminated cells be observed with naked eyes? What are the effects of mycoplasma contamination on the cell culture?**
   Mycoplasma can influence the growth parameters and metabolism of the infected cells. But the abnormal state of mycoplasma contaminated cells cannot be observed with naked eyes. Therefore, the cells must be verified as mycoplasma-free before the experiment, or the experimental results have no reference value.

8. **Why the color of medium become dark red when store at 4°C?**
   At 4℃, the release of CO_2 from the medium gradually results in the increase of the pH value of the medium. And phenol red is usually added to the medium, so the color of the medium will appear dark red with the increase of pH value. The culture medium with a high pH value may result in cell growth stagnation or death. In such case, aseptically filtered HEPES solution can be added to adjust the pH.

9. **When should the medium be changed?**
   This depends on the density or the growth state of the cells. When the cells are not in good condition or the cells grow slowly, it is recommended to change the medium (the medium of suspension cells must be centrifuged before changing, and the medium of adherent cells can be directly replaced), or supplement fresh medium directly to stimulate cell growth. For adherent cells, they can be washed with PBS to remove cells that in poor condition, then add fresh medium.).

10. **What is the concentration of EDTA-trypsin- when passaging adherent cells? What is the time for trypsinization of cells?**
    The frequently-used concentration of cell dissociation reagent is 0.25% EDTA-trypsin (if the cells are not resistant to dissociation or sensitive to trypsin, please halve the concentration of trypsin). It is recommended to wash the cell layer with PBS before passaging the cells to remove the serum in residual medium (residual serum will affect the activity of trypsin) and subsequently treated the cells with EDTA-trypsin- solution. For first cultured cells or for beginners with no cell culture experience, it is recommended to observe the cells after treat with trypsin under inverted microscope. When 80% of the cells detached, add medium containing serum to stop the trypsinization of trpsin.

11. **How to avoid the adherent cells to grow in cluster?**
    The most important reason for cell cluster growth is that the cells are not well-distributed during subculture process, which is mainly caused by the following two reasons: (1) The

trypsinization time is not enough for sufficient cell disassociation, thus the cells are detached by pieces from the surface of cell culture vessel. (2) Cells are not evenly distributed when resuspended after centrifugation. Therefore, it is proper to control the trypsin disassociation time and ensure that cells are distributed evenly during resuspension.

12. How to prepare cell freezing medium?

The components of cell freezing medium are basic medium, serum (20%-90%) and DMSO (no more than 10%). The commonly-used freezing medium in laboratories contains 70% basic medium, 20% serum and 10% DMSO. Note: Because of the dramatic heat generation when the DMSO is added to medium and serum, the prepared cell freezing medium should be pre-cooled before use.

13. How to cryopreserve the cells?

Cryopreservation method 1: place the cryovials containing the frozen cells at 4°C for 30 minutes → at -20°C for 30 minutes → at -80°C for 16~18 hours →put into liquid nitrogen for long-term storage. (Note: It should be less than 1 hour at -20°C to prevent large ice crystals from causing mass cell death.)

Cryopreservation method 2: pre-cool the commercial programmable cooler at 4°C in advance → place the cryovials containing the frozen cells in a programmable cooler and then placed in a –70°C to –90°C freezer for overnight → transfer the cryovials to liquid nitrogen for long-term storage (this method is recommended due to its high cell survival after resuscitation).