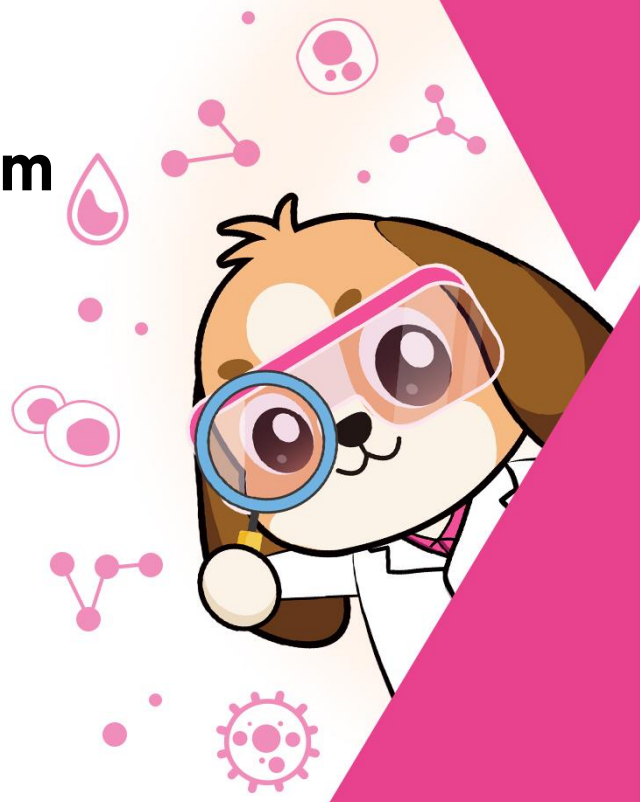
cyagen | OriCell™

User Manual

OriCell™ Serum Free Medium For Human Dental Pulp Mesenchymal Stem Cells (Type II, Phenol Red-Free)



Catalog No. HUXDP-90063

We help you discover life

Introduction

Developed and introduced by the OriCell™ team, the OriCell™ Serum Free Medium For Human Dental Pulp Mesenchymal Stem Cells (Type II, Phenol Red-Free) includes essential components such as a cell basal medium for nutrient supply and serum substitutes.

This product is specifically designed for the serum-free culture of human dental pulp mesenchymal stem cells, effectively maintaining robust proliferation rates while preserving their differentiation potential.

Note: **This product is only provided for further scientific research. It is not intended for diagnostic, therapeutic, clinical, household, or any other applications.**

When citing our products in academic journals, please indicate "OriCell™ + Catalog Number, from Cyagen Biosciences (Guangzhou) Inc."

Product Information

Components	Catalog Number	Volume
OriCell™ Basal Medium For Cell Culture	BLPF-03011	475 mL
OriCell™ Supplements For Human Dental Pulp Mesenchymal Stem Cells Serum Free Culture	HUXDP-04063	25 mL

QC

- Pass the detection of bacteria, fungi, mycoplasma, and endotoxins.
- Pass the detection of osmotic pressure and pH.
- Pass the detection of product quality.

Please reference "COA" for details.

General Handing Principles

1. Ensure that all equipment is kept clean and tidy.
2. Standard operation method. Please operate according to the method described in the product manual, strictly control the variables, and do a controlled experiment.
3. The ingredients should be properly stored in accordance with the storage conditions and used as soon as possible.
4. If a complete set of medium cannot be used in a short period of time, it should be prepared in batches according to the volume ratio of each component in the kit and stored in aliquots.

Product Stability and Storage Conditions

1. All ingredients must be kept in dark place.
2. The basal medium must be stored in a refrigerator at 4°C for a period of 1 year. Other components must be stored at -20°C for a period of 2 years.

3. The prepared complete medium can be stored at 4°C for a period of 1 month. If the culture conditions are stable, the container has great sealing performance, and there is no alternation of hot and cold condition, the period of using can be appropriately extended, but not exceed 45 days.
4. Please use all products within the expiration date. Expired ingredients may significantly affect the cell culture effect.

Preparation of Complete Medium

Materials Required

- OriCell™ Serum Free Medium For Human Dental Pulp Mesenchymal Stem Cells (Type II, Phenol Red-Free) (Cat. No.: HUXDP-90063)
- Clean,terile,and stable quality disposable consumables (pipettes, pipette tips, centrifuge tubes, etc.)
- Clean sealing film
- Aluminum foil paper and other light-avoiding materials

Steps

1. At least 1-2 hours before preparation, place the OriCell™ Supplements For Human Dental Pulp Mesenchymal Stem Cells Serum Free Culture (Cat. No.: HUXDP-04063) in a refrigerator at 4°C to thaw completely.
2. Use 75% ethanol to carefully wipe the outer packaging of all ingredients. Open the package in the clean bench.
3. Add all the supplements to OriCell™ Basal Medium (Cat. No.: BLPF-03011).

   Note: **It is recommended that when the serum-free additive melts for the first time, it is divided into small branches and stored at -20°C to avoid repeated freezing and thawing. There may be a small amount of precipitate in the additive, which has no effect on cell culture.**
4. Tighten the cap of the basal medium bottle, shake gently and thoroughly.

5. Seal the bottle with parafilm, wrap the bottle with aluminum foil paper, and mark the name, preparation date and other information.

   Note: **All components in complete medium are strictly aseptically controlled. Under normal circumstances, we do not recommend sterilization again. If there is a risk of contamination during the preparation process, the complete medium can be filtered and sterilized. Please choose whether to add Penicillin/Streptomycin according to your own needs. If you do need, please buy it yourself.**

Cell Culture

Materials Required

- OriCell™ Serum Free Medium For Human Dental Pulp Mesenchymal Stem Cells （Type II, Phenol Red-Free） (Cat. No.: HUXDP-90063)
- OriCell™ Animal Protein-free Dissociation Solution (Cat. No.: APFD-10001)
- OriCell™ Phosphate-Buffered Saline Solution (1X) (Cat. No.: PBS-10001)
- Clean,terile,and stable quality disposable consumables (pipettes, pipette tips, centrifuge tubes, etc.)

Thawing and Establishing of Cells

1. Preheat the water bath at 37°C.
2. Warm the complete medium to 37°C.
3. Add more than 5 mL of complete medium to a 15 mL centrifuge tube for use.
4. Take the cells out of the -80°C refrigerator, put them in a 37°C water bath and shake them quickly to thaw the cryopreservation solution.

   Note: **During the thawing process, the cryotube must be shaken to ensure that the solution thaws quickly and evenly.**

5. When shaking, please avoid water immersing the pipe cover to cause pollution.

6. When the cryopreservation solution has thawed into ice crystal with a diameter of about 2 mm, stop the water bath. Continue to shake the cryotube until the ice crystal melts thoroughly.
7. Wipe the outer surface of the cryotube with 75% ethanol.
8. Open the cryopreservation tube in the ultraclean bench, use a Pasteur pipette to suck the cell suspension, and transfer it to the prepared centrifuge tube.
9. Wash the cryotube once with 1 mL of complete medium to collect residual cells to reduce loss.
10. Centrifuge the cell suspension at 250×g for 4 minutes.
11. Remove the supernatant after centrifugation. Add 2 mL of complete medium, gently pipette the cell pellet, blow and mix thoroughly.
12. Inoculate the cells into a suitable culture container at $(2.5\sim4)\times10^4$ live cells/cm^2, or adjust the passage ratio according to the actual density of the cells.
13. Shake the cells well and incubate them in a CO_2 incubator at saturated humidity, 37°C, 5% CO_2 inside.

    Note: **Do not move or observe the cells within 2 hours of inoculation. This will seriously affect cell adhesion, resulting in poor shape, cell clumping, and uneven adhesion.**
14. On the next day of recovery, observe the cell status, and replace medium with fresh complete medium or passage.
15. Then refresh the complete medium every 2 days until the cells have grown to 90% confluence, which requires passage generation.

Passaging of Cells

1. Prewarm the complete medium and trypsin to 37°C.
2. Remove the medium in the culture container.
3. Wash the cells twice with PBS (approximately 3 mL for T25 flask and 6 mL for T75 flask). Please perform relatively slightly and wash thoroughly. Remove the PBS.
4. Add trypsin (approximately 1.5 mL for T25 flask and 3 mL for T75 flask), spread quickly to ensure full contact with the cells.

    Note: **Due to the different titers of digestive fluids used in different laboratories, the digestion time**

may be slightly different, and the specific time should be based on the situation observed under the microscope; If common pancreatic enzymes are used, the recommended concentration is 0.05%.

5. Observe the cells under a microscope. After about 70%~80% of the cells have shrunk and round, tap the outer wall of the culture vessel to remove the cells from the culture surface.
6. Immediately add three times the volume of 1xPBS for dilution and digestion.
7. Use a pipette to suck up the cell suspension, pipetting the bottom surface of the culture container several times, and pipetting down as much as possible of the cells.

   Note: **The pipetting action should not be violent.**
8. Transfer the cell suspension to a centrifuge tube. Wash the container once with PBS (approximately 3 mL for T25 flask and 6 mL for T75 flask) to collect residual cells.
9. All the collected cell suspensions are centrifuged at 250×g for 4 minutes.
10. Remove the supernatant after centrifugation. Add 2 mL of complete medium, gently pipette the cell pellet, blow and mix thoroughly.
11. Inoculate the cells into a suitable culture container at $(2.5\sim4)\times10^4$ live cells/cm^2, or adjust the passage ratio according to the actual growth of the cells.
12. Shake the cells well and incubate them in a CO_2 incubator at saturated humidity, 37°C, 5% CO_2 inside.
13. Then refresh the complete medium every 2 days until the cells have grown to 90% confluence, which requires passage generation or frozen.

    Note: **Human dental pulp mesenchymal stem cells optimal subculturing should be initiated upon attaining 80-90% confluency to prevent contact inhibition-mediated growth arrest. Prolonged maintenance beyond this threshold may result in compromised proliferative capacity and altered morphological characteristics due to overcrowding-induced stress responses.**