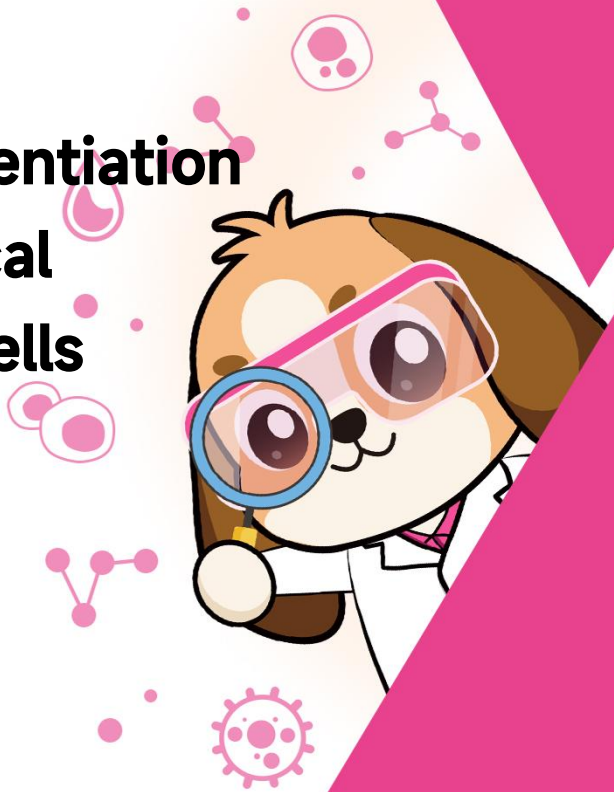


## User Manual

# OriCell™ Osteogenic Differentiation Medium For Human Umbilical Cord Mesenchymal Stem Cells

Catalog No. HUXUC-90021



## Introduction

OriCell™ Osteogenic Differentiation Medium For Human Umbilical Cord Mesenchymal Stem Cells, developed by the R&D team, contains a basic medium suitable for the growth of human umbilical cord mesenchymal stem cells, OriCell™ Premium Fetal Bovine Serum, and a supplement for osteogenic differentiation of umbilical cord mesenchymal stem cells.

This product is suitable for osteogenic induction and differentiation of human umbilical cord mesenchymal stem cells. Extensive cell culture data have demonstrated that this product can induce these cells to differentiate efficiently and stably into osteoblasts.

**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                                                         | BLDM-03011     | 177 mL |
| OriCell™ Fetal Bovine Serum (Superior-Quality)                                                 | FBSSR-01021    | 20 mL  |
| OriCell™ Supplement For Human Umbilical Cord Mesenchymal Stem Cells Osteogenic Differentiation | HUXUC-04021    | 3 mL   |
| Alizarin Red S Solution (pH=5.1~5.3)                                                           | ALIR-10001     | 10 mL  |
| Gelatin                                                                                        | GLT-11301      | 10 mL  |

## QC

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

Please refer to "COA" for details.

## General Handling Principles

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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.
3. The ingredients should be properly stored in accordance with the storage conditions and used as soon as possible.
4. If complete 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

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

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### Materials Required

- OriCell™ Osteogenic Differentiation Medium For Human Umbilical Cord Mesenchymal Stem Cells (Cat. No.: HUXUC-90021)
- Clean, sterile, 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 6 hours before preparation, place the OriCell™ Superior Fetal Bovine Serum (Cat. No.: FBSSR-01021) in a refrigerator at 4°C to allow it to thaw completely.  
**Note:** Flocs may appear in the thawed serum, the main component of fibrin, which will not affect the product performance. Unless the cell culture system is required for a high degree of purification, we do not recommend removing flocs.
2. At least 30 minutes before preparation, place the OriCell™ Supplement For Human Umbilical Cord Mesenchymal Stem Cells Osteogenic Differentiation in a refrigerator at 4°C until it is completely thawed.
3. Turn upside to down or flick the reagent tube to mix the reagent.

4. Carefully wipe the outer packaging of all ingredients with 75% ethanol. Open the package inside a clean bench.
5. Add all of the supplement (Cat. No.: FBSSR-01021) to OriCell™ Basal Medium (Cat. No.: BLD-03011).
6. Use a small volume of basal medium to rinse each bottle and tube, then add all the contents to the basal medium to maximize recovery.
7. Securely tighten the cap on the basal medium bottle and shake gently but thoroughly to mix.
8. Seal the bottle with Parafilm, wrap it in aluminum foil, and label it with the product name, preparation date, and other relevant information.

### Special Reminder

- If the medium will not be used up immediately, we recommend preparing in batches. Please prepare the required amount according to the ratio of each component in the kit. Any remaining components must be stored according to their respective storage conditions and should not be subjected to multiple freeze-thaw cycles.
- All components in the OriCell™ Osteogenic Differentiation Medium For Human Umbilical Cord Mesenchymal Stem Cells 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.
- The prepared osteogenic differentiation medium should be aliquoted into small portions to avoid repeated freeze-thaw cycles.

## Procedure for Inducing Differentiation

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### Materials Required

- OriCell™ Osteogenic Differentiation Medium For Human Umbilical Cord Mesenchymal Stem Cells (Cat. No.: HUXUC-90021)
- OriCell™ 0.1% Gelatin Solution (Cat. No.: GLT-11301)
- OriCell™ Phosphate-Buffered Saline Solution (1X) (Cat. No.: PBS-10001)
- OriCell™ Alizarin Red S Solution (Cat. No.: ALIR-10001)

### Steps

#### Note:

- 1) This protocol uses a 6-well plate as an example. Please select an appropriate culture vessel based on your specific needs.
  - 2) In order to avoid the cells floating, we recommended to use gelatin to coat the culture container.
  - 3) The induction medium needs to be preheated to 37°C before use.
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1. Add 1 mL of 0.1% gelatin solution to the 6-well plate and shake it completely cover the bottom of each well.
  2. Place the 6-well plate covered with 0.1% gelatin solution in the ultraclean bench or CO<sub>2</sub> incubator for at least 30 minutes.
  3. After 30 minutes, suck off the gelatin to inoculate cells, or wait for the 6-well plate to dry before inoculation.
  4. Inoculate the Human Umbilical Cord mesenchymal stem cells in the 6-well plate at a cell density of  $2 \times 10^4$  cells/cm<sup>2</sup>, and add 2 mL of ordinary complete medium to each well.
  5. The cells are cultured in a CO<sub>2</sub> incubator at 37°C, 5% CO<sub>2</sub>, with saturated humidity.
  6. When the cell density reaches 70%, please add 2 mL OriCell™ Osteogenic Differentiation Medium to the 6-well plate carefully.

7. Replace with the fresh medium every 3 days.
8. After 2 to 4 weeks of induction, stain with OriCell™ Alizarin Red S Solution (Cat. No.: ALIR-10001) depending on the morphological changes and growth of the cells.

**Note:** To prevent the loss of osteoblasts and calcium nodules, we recommended to change the fluid in half each two days after obvious calcium nodules could be found in the process of osteogenesis.

## Alizarin Red Staining Analysis

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### Materials Required

- OriCell™ Phosphate-Buffered Saline Solution (1X) (Cat. No.: PBS-10001)
- 4% Paraformaldehyde or 10% Formalin
- OriCell™ Alizarin Red S Solution (Cat. No.: ALIR-10001)

### Steps

**Note:**

- 1) To prevent calcium nodules falling off, all operations should be performed as gently as possible.
  - 2) Bring OriCell™ Alizarin Red S Solution to room temperature before use. If staining is insufficient, the staining time can be extended accordingly.
  - 3) Make sure calcium nodules appear before performing staining.
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1. After the osteogenic differentiation is completed, aspirate the complete osteogenic differentiation medium in the 6-well plate, and wash with 1×PBS for 2~3 times gently.
  2. Add 2mL of 4% Paraformaldehyde (or 10% Formalin) to each well and fix cells at room temperature for 30 minutes.
  3. Aspirate the fixative and wash with 1×PBS 2~3 times gently to ensure that the fixative is thoroughly washed.

4. Add 2mL of OriCell™ Alizarin Red S Solution (Cat. No.: ALIR-10001) to each well and incubate for 5~10 minutes at room temperature.
5. Aspirate the solution and wash with 1×PBS for 2~3 times to fully wash off the excess staining solution.
6. Add 2mL 1×PBS to each well and place the culture plate under a microscope to observe the results of osteogenic staining.
7. After the stained 6-well plate is encapsulated with parafilm, it can be stored at 4°C for 2 weeks.

### The Effect of Alizarin Red Staining



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