

# Human Umbilical Cord Mesenchymal Stem Cells Chondrogenic

## Induction Differentiation Medium

**Cat:** D3524

**Size:** 100mL/200mL

**Storage:** The basic medium should be stored in the refrigerator at 4°C, other ingredients should be stored at -20°C, valid for 1 year.

### Introduction:

Human umbilical cord mesenchymal stem cells chondrogenic induction differentiation medium is specially developed for human umbilical cord mesenchymal stem cells chondrogenic induction differentiation, optimize the formulation of differentiation reagents according to the characteristics of human umbilical cord mesenchymal stem cells, can increase the chondrogenic differentiation effect of human umbilical cord mesenchymal stem cells. This product contains serum components and is intended for scientific research purposes only, not for diagnosis, treatment, clinical or other purposes.

### Kit Components:

Chondroblast induced differentiation medium:

Kit Components	Add volume-100mL	Add volume-200mL
Basal Medium For Stem Cells Chondrogenic Induction Differentiation	87.6mL	175.2mL
Fetal Bovine Serum For Stem Cells Chondrogenic Induction Differentiation	10mL	20mL
Supplement For hMSCs Stem Cells Chondrogenic Induction Differentiation	2.4mL	2.4mL×2

Auxiliary reagent:

Kit Components	Add volume-100mL	Add volume-200mL
Alcian Blue 8GX Solution	10mL	10mL×2
Gelatin Solution	10mL	10mL×2

Note: Each component should be stored according to the temperature indicated on the label on the reagent tube.

### Protocols(only for reference):

1. Preparation of human umbilical cord mesenchymal stem cells chondrogenic induction differentiation medium

- (1) Preface: This product is kit type. Before use, it is necessary to mix each component reagent in the kit.
- (2) Preparation: defrost the serum at 4°C until it is completely melted; Defrost the additive at room temperature until completely melted, shake gently and mix well.
- (3) Configuration [Serum-additives]: Mix the serum and additives and shake them well. Divide them into several parts and freeze them at -20°C.
- (4) Complete configuration: Add 1 volume of [serum-additive] to 7 volume of basic medium, mix well for marking, and then use.

Note: The prepared chondrogenic induction and differentiation medium must be used on the same day.

## 2. Human umbilical cord mesenchymal stem cells chondrogenic induction differentiation operation guidance

- (1) When the fusion degree of your human umbilical cord mesenchymal stem cells reaches 80-90%, it can be digested with 0.25% pancreatic enzyme.
- (2) The digested human umbilical cord mesenchymal stem cells were counted, and according to the counting results, the grafted cells were added to chondroblast induced complete media at the rate of  $7.5 \times 10^5$  cells/mL, and then centrifuged 150g for 5min;
- (3) Discard the supernatant and add it into the chondroblast induced differentiation complete medium (ready for use) at the amount of  $5.0 \times 10^5$  cells/mL, and then reinsert the cells.
- (4) 500 $\mu$ L cell suspension (i.e.  $2.5 \times 10^5$  cells) was absorbed and transferred into a 15mL centrifuge tube, centrifuged 150g for 5min;
- (5) Do not shake or blow the cell mass after centrifugation, and carefully loosen the centrifuge tube cover to facilitate gas exchange. Incubate in 37°C, 5% CO<sub>2</sub>.

Note: Do not shake the centrifuge tube for 48 hours and keep the centrifuge tube standing.

- (6) After 48h, change the cartilage differentiation induction solution every 2 to 3 days, 0.5mL per tube, and flick the cell mass to make it float off the wall.
- (7) Unscrew the tube cap and put it in 37°C, 5% CO<sub>2</sub> for further induction; During induction, the diameter of the cell mass will increase and the surface will become smooth and gelatinous.

Note: To make it easier for the cells to coalesce into a ball, choose a centrifuge tube with a rounder bottom.

- (8) After 20 to 30 days of continuous induction, the cartilages can be sectored with neutral formaldehyde and paraffin embedding. After secting, staining identification can be performed according to the needs of the experiment. (This kit provides Alcian Blue 8GX Solution dye solution)

Note: Alcian Blue 8GX Solution also known as Alcian Blue 8GX Solution.

## 3. Alcian Blue 8GX Solution staining analysis

- (1) Fixation: neutral formaldehyde fixation, paraffin embedding section.
- (2) Dyeing steps:
  - 1) Dewaxing paraffin sections to water, rinse with distilled water. (Do not rinse the tissue directly, in case the slice is removed and the tissue is damaged);
  - 2) Soak Alcian Blue dye for 30min;
  - 3) Rinse with running water for 2min;
  - 4) distilled water to stop dyeing;
  - 5) Observe the degree of staining under a microscope and take photos.
- (3) Interpretation of the result: the cartilage and acidic mucus were blue.

### Notes:

1. Because there are more components in the medium, please pay strict attention to aseptic operation in the preparation process; If you are worried about the bad operation in the mixing process, please carry out 0.22 $\mu$ m filter membrane filtration on the complete medium after mixing the reagent.
2. Chondrogenic differentiation additives contain cell growth factors, remember to use now, prepared complete media must be used on the same day.