H UCMSCs Chondrogenic differentiation medium kit (ready-to-use)

Catalog No.: RC0140

Product Introduction

This product is an Chondrogenic differentiation medium kit H UCMSCsinto chondrogenesis. All products in the kit have passed biosafety testing and product quality testing with stable and effective performance.

Basic Information

Chondrogenic Differentiation Medium -Premix

Kit Components	Specification	Storage	Validity
H UCMSCs Chondrogenic Differentiation Premixed Medium (Contains Inducible Factors)	99 mL	2~8℃, dark	**3 months**

Chondrogenic differentiation medium - induction medium

Kit Components	Specification	Storage	Validity
TGF-β3	1 mL	-20 ℃	12 months

Note: Add 10 μL of TGF-β3 to every 1 mL of premix solution. Mix well to obtain the induction medium. Prepare it freshly and use it within 12 to 24 hours. It is recommended to aliquot TGF-β3 for use.

Staining solution

Kit Components	Specification	Storage	Validity
Alcian Blue Solution	5 mL	2~8℃, dark	12 months

Quality inspection standards

pH: 7.2~7.4
Endotoxin content: <10 EU/mL
Biosafety: Negative for bacteria, fungi, and mycoplasma
Purpose: Enhancing the ability of H UCMSCsto induce differentiation into chondrogenesis

Principle

Alecin blue is widely used for staining acidic polysaccharides, such as glycosaminoglycans in cartilage or tissues and polysaccharides secreted by cells. Under the action of induction culture medium, stem cells will gradually differentiate into chondrocytes. There is a layer of proteoglycan-rich matrix outside the chondrocytes, which is a marker of chondrogenic differentiation and can be stained blue-green by Alecin blue.

Instructions

Chondrogenic differentiation procedure (planar induction)

1. Cell differentiation induction

The cells in the logarithmic growth phase were digested and counted, and the cells were resuspended in chondrogenic differentiation medium. After centrifugation, the cell density was adjusted to $1.0\sim2.0\times10^7$cells/mL.

Pipette 20 μL of cell suspension (about $2.0\sim4.0\times10^5$ cells) and drop it in the center of a 24-well plate. Incubate at 37°C, 5% CO_2 for 2~3 h to allow cells to adhere.

After 2-3 hours, add 1mL of chondrogenic differentiation medium and culture normally. Change the medium every 2-3 days. Induce for 21-28 days according to the above medium change frequency, and pay attention to observe the changes in cell morphology.

2. Staining Identification

2.1 Cell fixation

Aspirate the culture medium and wash once with an appropriate amount of 1×PBS. Discard the solution and take an appropriate amount of 4% neutral formaldehyde solution to cover the bottom of the culture dish. Fix at room temperature for 30-60 min, discard the fixative and wash twice with 1×PBS.

2.2 Alecian blue staining

Add an appropriate amount of staining solution to the cleaned induction wells and leave to stain for 30 min away from light.

Aspirate the Alcian blue staining solution, wash twice with 1× PBS, and add an appropriate amount of 1× PBS to prevent the cells from drying out.

2.3 Induction evaluation

The cartilage staining effect was observed under a microscope, and image acquisition and induction evaluation were performed. When the induction was successful, the acidic mucopolysaccharide in the cartilage tissue could be stained blue-green by alexin blue.

Chondrogenic differentiation procedure (three-dimensional culture)

1. Preparation of stem cells

Digest and count the cells in the logarithmic growth phase. Transfer 3 × 10^5 cells to a 15mL centrifuge tube and centrifuge at 250g for 4 minutes.

Discard the supernatant, add 0.5 mL of chondrogenic differentiation medium premix, resuspend the cells, and centrifuge at 150g for 5 min. Discard the supernatant carefully, add 0.5mL of chondrogenic differentiation medium premix, resuspend the cells, and centrifuge at 150g for 5 min.

Slightly unscrew the cap of the 15 mL centrifuge tube and place it in a 37°C, 5% CO_2 culture environment for incubation.

2. Cell differentiation induction

After 24h, observe the deformation and aggregation of the cell pellet. If there are obvious changes, carefully and gently move the bottom of the tube to try to get the cell clusters off the bottom of the tube and completely immerse them in the induction solution.

Culture at 37°C, 5% CO_2 for about 21 days. Usually, replace the freshly prepared chondrogenic differentiation medium every 2 days. Pay attention to the sphering of cell clusters and surface smoothness, decide when to terminate cell induction, and perform staining identification.

3. Staining Identification

3.1 Chondrosphere fixation

The chondrosphere were transferred from the centrifuge tube to an EP tube, washed twice with 1× PBS, and finally placed in an appropriate amount of 4% neutral formaldehyde solution.

3.2 Paraffin-embedded sections

The chondrosphere were embedded in paraffin and then sectioned.

3.3 Alecian blue staining

The paraffin sections were dewaxed and dehydrated, stained with Alecin blue staining solution for 30 min, rinsed with tap water for 2 min, and rinsed once with distilled water.

3.4 Induction Evaluation

The cartilage staining effect was observed under a microscope, and image acquisition and induction evaluation were performed.

Acidic mucopolysaccharides can be stained blue-green by alicein blue.

NOTE: The level of chondrogenic differentiation of stem cells varies depending on factors such as cell type, cell donor source, culture conditions, cell passage, cell state, and differentiation time.

Precautions

1. The components of this product are all sterile and can be directly prepared into complete culture medium; the staining solution is an independently packaged component and should not be mixed with the culture medium
2. preparing the induction solution, please centrifuge each tube of small-dose reagent instantly to avoid loss. After preparation, please use it within the validity period.

3. premixed solution: 2~8 ℃, protected from light; the validity period after preparation is 3 months. The induction solution should be prepared and used immediately.
4. This product is only for scientific research experiments and cannot be used for clinical treatment.