Chondrogenic Differentiation Small molecule compound Kit-3 (Sterile Solution, With-Alcian blue)

Cat: IK-CHD-3
Storage: -20℃, 6 months

Introduction

This Kit is a small molecule compound kit designed by Solarbio for the induction and differentiation of chondrocytes. It does not contain TGF-β, and customers need to prepare appropriate TGF-β separately. This Kit selected five classic basic reagents (sterile) and high-quality alixin blue staining solution (pH 2.5) for adipogenic induction, aiming to build a ' one-stop service platform ' for customers, saving time and effort. The products in the library have passed the biosafety test and product quality test, with stable and effective performance, small batch difference, good biological activity, and a large number of literatures have been verified in many ways, and the quality is reliable.

Alcian blue (Alcian) is a copper-titanium-like conjugated dye. When the pH value is 2.5, the carboxyl group in the tissue is ionized, with a negative charge, forming a salt bond with the cations in Alcian blue to stain the tissue with carboxyl groups (such as proteoglycan / hyaluronic acid and epithelial acidic mucin). Neutral mucins (such as neutral mucins in gastric mucosa and Brunner gland) could not react with alcian blue. Alcian blue staining solution (pH 2.5) is widely used in the staining of acidic polysaccharides, such as glycosaminoglycans in cartilage or tissues and extracellular polysaccharides secreted by cells. Stem cells will gradually differentiate into chondrocytes under the action of induction medium. There is a layer of proteoglycan-rich extracellular matrix, which is a marker of chondrogenic differentiation and can be stained blue-green by alixin blue.

Kit Components

Kit components	Size	Storage
Reagent 1 10mM dexamethasone	50μL	-20℃
Reagent 2 25 mg / mL vitamin C	2*300μL	-20℃
Reagent 3 100 mg / mL L-proline	120μL	-20℃
Reagent 4 100 × ITS Supplement	1mL	2-8℃
Reagent 5 1M sodium pyruvate	120μL	-20℃
Reagent 6 alcian blue staining solution (pH 2.5)	10mL	RT

Note

a. Reagent 1, Reagent 2, Reagent 3, Reagent 4 and Reagent 5 are all sterile solutions, which can be

directly added to the medium for use.

b. Vitamin C is more unstable after dissolution. It is recommended to freeze it by self-packaging (avoid repeated freezing and thawing). When changing the liquid, take out a thaw and use it. This Kit gives an additional reagent two.

c. Reagent 4 is an animal-free component with a concentration of 1.0 mg/mL insulin, 0.550 mg/mL transferrin and 0.67 μg/mL sodium selenite.

d. Reagent 6 is the subsequent independent verification experimental component, please do not mix with the culture medium.

e. Before use, please immediately centrifuge each tube of small doses of reagents to avoid loss.

f. This product is for scientific research only. Do not use for medicine, clinical diagnosis or treatment, food and cosmetics. Do not store in ordinary residential areas.

g. For your safety and health, please wear a good lab coat and wear disposable gloves and masks.

Product Features

1. This Kit is a universal chondrogenic induction small molecule compound kit without TGF-β, and customers need to prepare appropriate TGF-β separately.
2. This Kit selected several classic small molecule compounds for chondrogenic induction and was equipped with Alcian blue staining solution.
3. This Kit is a ready-to-use Kit, which can be used directly without further preparation or sterilization by customers.
4. The company provides TGF-β of different species and types. Customers can contact our company to purchase and customize the exclusive cartilage Kit.

Protocols *(only for reference)*

The level of chondrogenic differentiation of stem cells varies with cell type, cell donor source, culture conditions, cell passages, cell status and differentiation time. The following methods are for reference only, and customers need to adjust according to the actual induction situation.

Chondrogenic differentiation induction operation (plane induction)

1.Cell differentiation induction

The cells in the logarithmic growth phase were digested and counted. The cells were resuspended in chondrogenic differentiation medium I (containing TGF-β, dexamethasone, vitamin C, L-proline, 1×ITS Supplement, sodium pyruvate). After centrifugation, the cell density was adjusted to 1.0 ~ 2.0×10^7 cells/mL.

A total of 20 μL cell suspension (about 2.0~4.0 ×10^5 cells) was dropped to the center of the 24-well plate. The cells were cultured at 37°C, 5% CO_2 for 2 ~3 h to make the cells adherent.

After 2 ~ 3 h, 1 mL chondrogenic differentiation medium I (containing TGF-β, dexamethasone, vitamin C, L-proline, 1 × ITS Supplement, sodium pyruvate) was supplemented for normal culture. The liquid was changed every 2 ~ 3 days. The cells were induced for 21-28 days according to the above frequency of medium change, and the morphological changes of the cells were observed.

2.Staining identification

Wash the medium with an appropriate amount of PBS once and discard PBS;

appropriate amount of 4% neutral formaldehyde solution was used to cover the bottom of the culture vessel, and the fixing solution was discarded after 30~60 min at room temperature.

Wash twice with PBS;

Add an appropriate amount of alcian blue staining solution to the clean induction hole and stand for 30 min in dark.

The staining solution was sucked and washed twice with PBS, and an appropriate amount of PBS was added to avoid cell drying.

Induction evaluationThe cartilage staining effect was observed under a microscope, and image acquisition and induction evaluation were performed. When the induction is successful, the acid mucopolysaccharide in the cartilage tissue can be dyed blue-green by alixin blue.

Chondrogenic differentiation induction operation (three-dimensional culture)

1.Preparation of stem cells

The cells in the logarithmic growth phase were digested and counted, and 3×10^5 cells were transferred to a 15 mL centrifuge tube, and 250 g was centrifuged for 4 min.

The supernatant was discarded and 0.5 mL chondrogenic differentiation medium II (containing dexamethasone, vitamin C, L-proline, 1 × ITS Supplement, sodium pyruvate, without TGF-β) was added. The cells were resuspended and centrifuged at 150 g for 5 min.

The supernatant was carefully discarded, and 0.5 mL of chondrogenic differentiation medium I (containing TGF-β, dexamethasone, vitamin C, L-proline, 1 × ITS Supplement, sodium pyruvate) was added. The cells were resuspended and centrifuged at 150 g for 5 min.

The lid of the 15 mL centrifuge tube was slightly opened and placed in a 37°C, 5 % CO_2 culture environment.

2.Cell differentiation induction

After 24 h, the cell precipitation deformation and agglomeration were observed. If there were obvious changes, the bottom of the tube was gently moved carefully, and the cell mass was tried to leave the bottom of the tube and all infiltrated in the induction fluid.

The cells were cultured at 37°C, 5% CO_2 for about 21 days, and the freshly prepared chondrogenic differentiation medium I (containing TGF-β, dexamethasone, vitamin C, L-proline, 1 × ITS Supplement, sodium pyruvate) was usually replaced every 2 days. Pay attention to the observation of cell pellet formation and surface smoothness, determine the termination of cell induction time, and staining identification.

3.Staining identification

Cartilage ball fixationThe cartilage balls were transferred from the centrifuge tube to the EP tube, washed twice with PBS, and placed in an appropriate amount of 4% neutral formaldehyde solution.

Cartilage balls were sliced after paraffin embedding.

Paraffin sections were dewaxed and dehydrated, stained with Alcian blue staining solution for 30 min, and rinsed with running water for 5 min.

Induction evaluationThe cartilage staining effect was observed under a microscope, and image acquisition and induction evaluation were performed. When the induction is successful, the acid mucopolysaccharide in the cartilage tissue can be dyed blue-green by alixin blue.

Related Products

IK-LIN-7 Adipogenesis Induces Kit-7 (Instant Form, Containing Saturated Oil Red O)

IK-OIN-5 Osteogenic Induction Small Molecule Kit-5 (ready-to-use, containing alizarin red dye and 10% CPC)