Chondrogenic Differentiation Small molecule compound Kit （Non-sterile Powder, With-Alcian blue)

Cat:IK-CHD-1

Storage: -20℃, 6 months

Introduction

This Kit is designed by Solarbio for chondrocyte induction and differentiation of small molecule compounds, does not contain TGF-β, the customer needs to prepare the appropriate TGF-β. This Kit selects five classic basic reagents for lipid induction and high-quality Alisin Blue Staining Solution (pH 2.5), aiming to build a “one-stop service platform” for customers. The aim is to build a “one-stop service platform” for customers, saving time, effort and worry. The products in stock have passed biosafety and product quality tests, with stable and effective performance, small batch-to-batch variations, good biological activity, and have been verified by a large number of literature, so their quality is reliable. Alcian is a copper-titanium anthocyanine-conjugated dye. At pH 2.5, carboxyl groups in tissues ionize to a negative charge and form salt bonds with the cations in Alcian, staining tissues with carboxyl groups (e.g., proteoglycans/hyaluronic acid and epithelial acidic mucins). Neutral mucins (e.g., in the gastric mucosa and Brunner's glands) do not react with Alisin Blue. Alisin Blue Staining Solution (pH 2.5) is widely used for staining acidic polysaccharides, such as glycosaminoglycans in cartilage or tissues and cell-secreted periplasmic polysaccharides. Stem cells will gradually differentiate towards chondrocytes in the presence of induction medium. Chondrocytes have an outer layer of proteoglycan-rich matrix, a marker of chondrogenic differentiation, which can be stained blue-green by Alisin blue.

Kit Components

Kit components	Size	Save
Reagent I Dexamethasone	5mg	2-8℃
Reagent II Vitamin C	20mg	2-8℃
Reagent III L-Proline	20mg	2-8℃
Reagent IV 100× ITS Supplement	1mL	2-8℃
Reagent V Sodium pyruvate	20mg	2-8℃
Reagent VI Alisin Blue Stain（pH2.5）	10mL	RT

Note

a. Reagent I, Reagent II, Reagent III and Reagent V are non-sterile packages, please filter and remove bacteria before use.

b. Reagent IV is a sterile solution without animal source, the components are 1.0 mg/mL insulin, 0.550 mg/mL transferrin and 0.67 μg/mL sodium selenite.

c. Reagent VI is a subsequent independent validation experiment component, please do not mix with culture medium.

d. Before use, please centrifuge each tube of small dose reagent instantaneously to avoid loss.

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

f. For your safety and health, please wear a lab coat and disposable gloves and mask.

Product Features

1.This Kit is a general-purpose small molecule compound kit for chondrogenic induction without TGF-β. Customers need to prepare suitable TGF-β separately.

2. This Kit is made of several classical small molecule compounds used for chondrogenic induction, and equipped with Alisin Blue staining solution.

3. All components of this Kit are in powder format or stock solution with long potency period, so that customers can flexibly plan their experiments and prepare the required concentration.

4. Our company provides different species and types of TGF-β. Customers can contact us to customize the exclusive cartilage-forming Kit after purchasing separately.

Protocols (*only for reference***)**

The level of chondrogenic differentiation of stem cells varies depending on cell type, cell donor source, culture conditions, cell generation, cell status and differentiation time, etc. The following method is for reference only, and customers need to adjust it according to the actual induction situation.

Chondrogenic induced differentiation operation (planar induction**)**

1. Induction of cell differentiation

Cells in the logarithmic growth phase were digested down for counting, and cells were resuspended into Chondrocyte Induced Differentiation Medium I (containing TGF-β, dexamethasone, vitamin C, L-proline, 1× ITS Supplement, and sodium pyruvate), and cell density density was adjusted by centrifugation to 1.0~2.0 x 10^7cells/mL.

Aspirate 20 μL of cell suspension (about 2.0~4.0 x 10^5 cells) suspension drop to the center of 24-well plate. Place in 37℃, 5% CO_2 culture environment for 2~3 h to make the cells adherent to the wall.

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

2. Stain identification

Aspirate the culture medium and wash it once with appropriate amount of PBS, discard the PBS;

Take appropriate amount of 4% neutral formaldehyde solution to cover the bottom surface of the culture vessel and fix it at room temperature for 30~60 min, then discard the fixing solution;

Wash the wells twice with PBS;

Add appropriate amount of Alisin Blue Staining Solution into the cleaned induction wells, and leave it to stain for 30 min, protected from light;

The staining solution was aspirated and washed twice with PBS, and appropriate amount of PBS was added to avoid drying of cells;

Induction assessment Microscopic observation of the effect of chondrogenic staining was performed with image acquisition and induction assessment. When induction is successful, the

endoacidic mucopolysaccharides in cartilage tissue can be stained blue-green by Alisin blue.

Chondrogenic induced differentiation operation (three-dimensional culture**)**

1. Preparation of stem cells

Cells in the logarithmic growth phase were digested down and counted, and 3×10^5 cells were transferred to a 15 mL centrifuge tube and centrifuged at 250 g for 4 min. Discard the supernatant, add 0.5 mL of chondrogenic differentiation medium II (containing dexamethasone, vitamin C, L-proline, 1× ITS Supplement, sodium pyruvate, no TGF-β), resuspend the cells, and centrifuge at 150 g for 5 min. Carefully discard the supernatant, add 0.5 mL of chondrogenic induction and differentiation medium I (containing TGF-β, dexamethasone, vitamin C, L-proline, 1× ITS Supplement, sodium pyruvate), resuspend the cells, and centrifuge at 150 g for 5 min. The caps of the 15 mL centrifuge tubes were slightly unscrewed and placed in 37°C, 5% CO_2 culture environment.

2. Induction of cell differentiation

After 24 h, the cells were observed to precipitate deformed agglomerates, and if there were significant changes, the bottom of the tube was carefully and gently rattled to try to get the cell mass to detach from the bottom of the tube and fully immerse in the induction solution.

The cells were placed in 37°C, 5% CO_2 culture environment for about 21 days, and the freshly prepared chondrogenic induction and differentiation medium I (containing TGF-β, dexamethasone, vitamin C, L-proline, 1× ITS Supplement, and sodium pyruvate) was usually changed every 2 days. Attention was paid to the observation of cell mass formation and surface smoothness to decide the time to terminate cell induction, and staining was performed for identification.

3. Stain identification

a. Chondrospheres were fixed by transferring chondrospheres from centrifuge tubes to EP tubes, which were washed twice with PBS and placed in an appropriate amount of 4% neutral formaldehyde solution.
b. The cartilage spheres were sectioned after paraffin embedding.
c. The paraffin sections were deparaffinized and dehydrated, stained using Alisin blue staining solution for 30 min, and rinsed with running water for 5 min.
d. Induction assessment The effect of staining into cartilage was observed microscopically, and image acquisition and induction assessment were performed. When induction was successful, the endoacidic mucopolysaccharides in cartilage tissue could be stained blue-green by Alisin Blue.