

# Chondrogenic Differentiation Small molecule compound Kit-2 (Non-sterile Powder, Without-Alcian blue)

Cat: IK-CHD-2

Storage: 2-8°C, 1 year

## Introduction

This Kit is a small molecule compound kit designed by Solarbio for the induction and differentiation of chondrocytes. It does not contain TGF- $\beta$ , and customers need to prepare appropriate TGF- $\beta$  separately. This Kit selects five classic basic reagents that can be used 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 detection and product quality detection, 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.

# **Kit Components**

| Kit components                | Size | Storage |
|-------------------------------|------|---------|
| Reagent 1 dexamethasone       | 5mg  | 2-8°C   |
| Reagent 2 vitamin C           | 20mg | 2-8°C   |
| Reagent 3 L-proline           | 20mg | 2-8°C   |
| Reagent 4 100× ITS Supplement | 1mL  | 2-8°C   |
| Reagent 5 Sodium pyruvate     | 20mg | 2-8°C   |

#### Note

- a. Reagent 1, reagent 2, reagent 3 and reagent 5 are all non-sterile packaging. Please filter and remove bacteria before use.
- b. Reagent 4 is a sterile solution without animal source, which is composed of 1.0 mg/mL insulin, 0.55 mg/mL transferrin and 0.67 μg/mL sodium selenite.
- c. Before use, please immediately centrifuge each tube of small doses of reagents to avoid loss.
- d. This product is only for scientific research. Do not use in medicine, clinical diagnosis or treatment, food and cosmetics. Do not store in ordinary residential areas.
- e. 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- $\beta$ , dexamethasone, vitamin C, L-proline, 1 × ITS Supplement, sodium pyruvate). After centrifugation, the cell density was adjusted to  $1.0\sim2.0\times10^7$  cells/mL.

A total of 20  $\mu$ L cell suspension ( about 2.0~4.0×10<sup>5</sup> cells ) was dropped to the center of the 24-well plate. The cells were cultured at 37°C, 5% CO<sub>2</sub> 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 d. The cells were induced for 21-28 d according to the above frequency of medium change, and the morphological changes of the cells were observed.

# 2. Alcian blue staining identification

# Chondrogenic differentiation induction operation (three-dimensional culture)

## 1. Preparation of stem cells

The cells in the logarithmic growth phase were digested and counted, and  $3 \times 10^5$  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,  $1 \times ITS$  Supplement, sodium pyruvate, without TGF- $\beta$ ) 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- $\beta$ , dexamethasone, vitamin C, L-proline,  $1 \times ITS$  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<sub>2</sub> 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<sub>2</sub> for about 21 d, and the freshly prepared



chondrogenic differentiation medium I (containing TGF- $\beta$ , dexamethasone, vitamin C, L-proline, 1  $\times$  ITS Supplement, sodium pyruvate) was usually replaced every 2 d. Pay attention to the observation of cell pellet formation and surface smoothness, determine the termination of cell induction time, and staining identification.

# 3. Alcian blue staining identification

## **Related Products**

| <i>IK-LIN-7</i> | Adipogenesis Induces Kit-7 (Instant Form, Containing Saturated Oil Red O)                              |  |
|-----------------|--|--|
| IK-OIN-5        | Osteogenic induction of small molecule compound Kit-5 (ready-to-use, containing alizarin               |  |
|                 | red staining solution and 10% CPC).  |  |
| IK-CHD-3        | Cartilage-induced small molecule compound Kit (i.e., containing alixin blue)                           |  |
| IKM1020         | $10 \times Protease$ and Phosphatase Inhibitor Cocktail (Universal type) IKM1010 $100 \times Protease$ |  |
|                 | Inhibitor Cocktail MIX (Versatility)   |  |
| IKC1032-1       | CEPT Cocktail Kit  |  |
| P02149          | Recombinant Human/Mouse/Rat TGF- $\beta$ 3/TGF-beta 3/TGFB3/Transforming Growth Factor $\beta$ -3      |  |
| P02078          | Recombinant Mouse/Rat TGF- $\beta$ 2/TGF-beta 2/TGFB2/Transforming Growth Factor $\beta$ -2            |  |
| P00121          | Recombinant Human TGF-beta 1/TGFB1   |  |