Osteogenic Inducible Small Molecule Compound Kit-1（Powder,With Alizarin Red S and CPC）

CAT NO.: IK-OIN-1

Storage:2-8℃，2 years

Introduction

This Kit is designed by Solarbio specifically for the induction differentiation of osteoblasts. This package features 3 classic basic reagents (powder size) and Alizarin Red S Standard、Alizarin Red S and CPC, all of which have good biological activity. It aims to build a "one-stop service platform" for customers, saving time, effort and worry. All the products in the library have passed biosafety testing and product quality testing, and have stable and effective performance, little difference between batches, and good biological activity. Moreover, there have been a lot of literatures and multi-party verification, and the quality is reliable.

Alizarin red S calcium staining is an authoritative and classical technique for the analysis of orange red calcium deposition in fixed cell samples by means of chelation technology, which makes calcium ions and alizarin red S produce complex. It is mainly suitable for the detection of calcium deposits and calcified nodules in animal protozoa or cultured cells. Under the influence of induction medium, stem cells will gradually differentiate into osteocytes, produce obvious calcium secretion reaction, and form calcium salt crystals or calcium nodules, which can be stained by alizarin red. There are orange or orange red deposits in the calcium salts, and the intensity of the color depends on the calcium salt content.

Kit Components

Reagent	Size	Storage
Reagent 1 Dexamethasone	5mg	2-8℃
Reagent 2 Vitamin C	10mg	2-8℃
Reagent 3 β-Glycerophosphate Sodium Salt Hydrate	500mg	2-8℃
Reagent 4 Alizarin Red S Standard	10mg	RT
Reagent 5 Alizarin Red S	120mg	RT
Reagent 6 CPC	1g	2-8℃

Note：

a. The components of this product are non-sterile, please filter sterilization before using reagents 1, 2 and 3.

b. Reagents 4, 5 and 6 are the subsequent independent verification test components, do not mix with the medium.

c. The prepared high concentration dexamethasone, Vitamin C, and β - glycerophosphate sodium stock solution is recommended to be filtered and sterilized. It is recommended to freeze the stock solution in several small portions to avoid repeated freezing and thawing (-20 ℃, 6 months).
d. Alizarin Red S and CPC can be directly formulated as working solution concentration and stored at 2-8℃ for 6 months away from light.
e. CPC can be dissolved by heating it in a water bath at around 50 ℃ to promote dissolution.
f. This product is only used for scientific research experiments, not for clinical treatment.

Product Characteristics

1. All products in this kit are packaged in powder form with a long shelf life, and can be freely prepared according to customer experimental needs.
2. Flexible customization. Customers can flexibly add or remove compounds (such as Alizarin Red, CPC, antibiotic, etc.) according to experimental needs. Customization of specifications and packaging is also possible.
3. Adequate inventory, spot delivery, high cost-effectiveness.

Protocols (*only for reference*)

1. Osteogenic Induction Differentiation (6-well plate as an example)

1.1 (optional) Gelatin Coated Culture Vessel

After the stem cell culture for a long time, it may appear to peel off the wall or float phenomenon, it is recommended to use 0.1% gelatin solution on the incubator.The dish is coated. Prepare a suitable culture vessel, take an appropriate amount of gelatin to cover the bottom, stand at 37°C for 30 min, absorb and dry before use.

1.2 Stem Cell Inoculation

The cells of logarithmic growth stage were inoculated into the coated culture vessel according to the cell density of 2×10^4cells/cm^2, and cultured at 37℃ and 5% CO_2 until the fusion degree was 60-70%. The supernant was discarded, and 2mL osteogenic induction differentiation medium (containing dexamethasone, vitamin C and β-Glycerophosphate Sodium) was added to each well.

1.3 Induction of Cell Differentiation

The cells were cultured at 37℃ and 5% CO2 for about 14 to 28 days, and the fluid was changed every 2 to 3 days, and the morphological changes of cells were observed. According to the precipitation of calcium salts and the formation of calcium nodules, the time of termination of cell induction was determined and staining was performed.

Note: If the curling is serious, half-change solution (only 1mL of fresh osteogenic induction differentiation medium is added each time) or half-change solution in the early stage and the late stage can be used for induction.

2. Dyeing Identification (6-well plate as an example)

2.1 Cell Fixation

Remove the culture medium and wash it once with an appropriate amount of 1×PBS. After discarding, take an appropriate amount of 4% neutral formaldehyde solution and cover the bottom surface of the culture vessel. Fixed at temperature for 30-60 min, discard the fixing solution, and then use 1×PBS to clean twice.

2.2 Alizarin Red Staining

Add alizarin red dye to each well for 3~5min, absorb the dye, wash twice with 1×PBS, and add an appropriate amount of 1×PBS to avoid cell drying.

Note: Dyeing time should not be too long, too long is easy to lead to calcium salt dispersion and poor coloring effect.

2.3 Induction Evaluation

The effect of osteogenic staining was observed under microscope, and image collection and induction evaluation were performed. Upon successful induction, the calcic nodules appear red or orange-red after binding with alizarin red dye.

Note: The level of osteogenic differentiation of stem cells varies depending on cell type, cell donor source, culture conditions, cell generation, cell state, and differentiation time.

2.4 Semi-Quantitative Analysis

After the microscopic observation, the supernatant was abandoned, an appropriate 10%CPC solution was added and incubated at room temperature for 15-60 min to dissolve the mineralized nodules (Alizarin red), and then OD value of the supernatant was detected at 562 nm.

Note: If OD value exceeds the range, the concentration of cetylpyridine chloride solution can be adjusted or the test sample can be diluted appropriately.

2.5 Standard Curve Drawing (taking enzyme marker as an example)

1) Preheat the ELISA for more than 30min, adjust the wavelength to 562 nm, and zero the distilled water.
2) Add 5mg alizarin red standard to 1mL distilled water to obtain 5mg/mL alizarin red standard liquid. It can be stored at 2-8℃ for 1 week. It was diluted with distilled water to a standard solution of 0.15625, 0.3125, 0.625, 1.25, 2.5, 5mg/mL concentration. (You can also set the standard range according to your own experimental needs)
3) Different concentrations of alizarin red standard liquid 20μL were added into 96-well plates, 180μL 10% cetylpyridine chloride solution was added, and fully mixed. OD value of supernatant was detected at 562 nm.
4) Take the concentration of each standard solution as the X-axis and ΔA (A standard tube -A blank tube) as the Y-axis to make a standard curve, and get the equation y=kx+b.

Related Literature

[1]. Zhang Z, et al. A drug-loaded composite coating to improve osteogenic and antibacterial properties of Zn-1Mg porous scaffolds as biodegradable bone implants. Bioact Mater. 2023 Apr 28;27:488-504.(IF: 18.9)

Related Products

IK-OIN-1 *Osteogenic Inducible Small Molecule Compound Kit-1*
IK-OIN-2 *Osteogenic Inducible Small Molecule Compound Kit-2*
IK-OIN-3 *Osteogenic Inducible Small Molecule Compound Kit-3*
IK-OIN-4 *Osteogenic Inducible Small Molecule Compound Kit-4*
IK-LIN-1 *Lipogenic Induction Of Small Molecule Compound Kit*
IK-CEA-1 *Cell Activation Kit-1*