Product Information

This product is an osteogenic differentiation medium kit H ADSCs into osteoblasts. All products in the kit have passed biosafety testing and product quality testing with stable and effective performance.

Basic Information

Kit Components	Specification	Storage	Validity
H ADSCsOsteogenic Differentiation Basal Medium	175 mL	2~8℃, dark	12 months
H ADSCs Osteogenic Differentiation FBS	20 mL	-20 ℃	24 months
P/S Solution Dual Antibody	2 mL	-20 ℃	12 months
Glutamine	2 mL	-20 ℃	12 months
β-Glycerophosphate	2mL	-20 ℃	12 months
Ascorbate Acid	400 μL	-20 ℃	12 months
Dexamethasone	20 μL	-20 ℃	12 months
Dye Liquor: Alizarin Red Solution	10 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 ADSCsto induce differentiation into osteoblasts

Principle

Alizarin Red is a widely used calcium indicator in biomedical sample testing. Free calcium ions do not form a red precipitate with Alizarin Red, whereas calcified nodules can be stained red. Under the influence of osteogenic induction medium, stem cells gradually differentiate into osteoblasts, producing a significant calcium secretion response, forming calcium salt crystals or calcified nodules, all of which can be stained by Alizarin Red.

Instructions

1. Osteogenic induction differentiation procedure
1.1 Gelatin coating of culture vessels
After a prolonged period of stem cell culture, detachment, curling, or floating may occur. It is recommended to coat the culture vessels with a 0.1% gelatin solution. Prepare the appropriate culture vessels, add an adequate amount of gelatin to cover the bottom, incubate at 37°C for 30 minutes, then aspirate and air-dry for use.
1.2 Inoculation of stem cells
Take cells in the logarithmic growth phase and inoculate them at a density of 2.0×10^4 cells/cm² onto the coated culture vessels. Cultivate them in a 37°C, 5% CO_2 environment until the confluence reaches 60-70%. Discard the supernatant and add osteogenic induction differentiation medium.
1.3 Cell differentiation induction
Cultivate in a 37°C, 5% CO_2 environment for approximately 14-21 days, changing the medium every 2-3 days, and observe the morphological changes of the cells. Determine the termination time of cell induction based on the precipitation of calcium salt crystals and the formation of calcified nodules, and proceed with staining identification.

2. Staining identification
2.1 Cell fixation
Aspirate the culture medium and wash once with an appropriate amount of 1×PBS. After discarding, cover

the bottom of the culture vessel with an appropriate amount of 4% neutral formaldehyde solution and fix at room temperature for 30-60 minutes. Discard the fixative and wash twice with 1×PBS.
2.2 Alizarin red staining
Add an appropriate amount of Alizarin Red staining solution and stain for 3-5 minutes. Aspirate the Alizarin Red staining solution, wash twice with 1×PBS, and add an appropriate amount of 1×PBS to prevent cell drying.
2.3 Induction assessment
Observe the osteogenic staining effect under the microscope, capture images, and assess the induction. When induction is successful, calcified nodules appear red or orange after binding with Alizarin Red dye.
Note: The level of osteogenic differentiation of stem cells varies due to factors such as cell type, donor source, culture conditions, cell passage number, cell state, and differentiation time.

Notes

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. Before preparing complete culture medium, please centrifuge each tube of small-dose reagent to avoid loss. After preparation, please use it within the validity period.
3. Storage conditions of complete culture medium: 2~8 ℃, protected from light; the shelf life is 3 months after preparation.
4. This product is only for scientific research experiments and cannot be used for clinical treatment.