Human Adipose Mesenchymal Stem Cells Osteogenic Induction Differentiation Medium

Cat: D3501
Size: 200mL
Storage: The basic medium should be stored in the refrigerator at 4℃, other ingredients should be stored at -20℃, valid for 1 year.

Introduction:

Human adipose mesenchymal stem cells osteogenic induction differentiation medium is specially developed for human adipose mesenchymal stem cell osteogenic induction differentiation. According to the characteristics of human adipose mesenchymal stem cells, optimize the formulation of differentiation reagents, which can increase the osteogenic differentiation effect of human adipose mesenchymal stem cells. This product contains serum components and is intended for scientific research purposes only, not for diagnosis, treatment, clinical or other purposes.

Kit Components:

Kit Components	Add volume
Human adipose mesenchymal stem cells induction differentiation basic medium	175mL
FBS	20mL
Glutamine	2mL
Penicillin-Streptomycin	2mL
β-Glycerophosphate	2mL
Ascorbate Acid	400μL
Dexamethasone B	200μL
Composition of dye solution:	
Alizarin red S dye solution	10mL

Note: Each component should be stored according to the temperature indicated on the label of the reagent tube. Dexamethasone A and dexamethasone B have different concentrations and should not be mixed.

Protocols(only for reference):

1. Preparation of human adipose mesenchymal stem cells osteogenic induction differentiation medium

(1) This product is kit type. Before use, it is necessary to mix each component reagent in the kit.

(2) Before use, please defrost the serum at 4℃ until it is completely dissolved; After the serum is completely dissolved, dissolve all additives at room temperature. After the reagent is completely dissolved, shake gently to mix the reagent well. (**Note:** In order to ensure the use of trace reagents, please centrifuge the reagent tube less than 200μL for a short time, so that all reagents can be collected to the bottom of the tube.)

(3) According to the above ingredient list, fully dissolved and mixed FBS, cyanstreptomycin, glutamine, β-sodium glycerophosphat, ascorbic acid and dexamethasone B were added into the induction base medium successively according to their volume; After the mixture is well marked, the medium can be used. (Sodium β-glycerophosphate has poor solubility and must be completely dissolved before being added to the induction medium)

Note: Absorb the reagent components in the sterile reagent tube, extend the gun head below the liquid surface of the medium, and gently blow and wash the gun head. Then absorb a small amount of the medium washing reagent tube, and add all components to the basic medium as completely as possible to ensure the effect of the medium.

2. Human adipose mesenchymal stem cells osteogenic induction differentiation operation guidance

This process requires preparation of human adipose mesenchymal stem cells complete medium, 0.25% pancreatic enzyme, 1×PBS and human adipose mesenchymal stem cells osteogenic induction differentiation medium. This operation guide takes the six-well plate as an example:

(1) When the fusion degree of human adipose-derived mesenchymal stem cells reaches 80~90%, it can be digested with 0.25% pancreatic enzyme.

(2) The digested human adipose-mesenchymal stem cells were counted and inoculated into the six-well plate according to the cell density of 2×10^4cells/cm^2(**according to the cell growth rate, the cell confluence reached 70%-80% on the second day after 6-well plate inoculation**). Each well was inoculated with 2mL of human adipose mesenchymal stem cells complete medium.

(3) The uniformly inoculated human adipose mesenchymal stem cells were cultured in an incubator at 37℃ and 5% CO_2.

(4) When the degree of cell fusion reached 70%~80%, the medium was carefully sucked out of the hole and 2mL of the medium for osteogenic induction differentiation of human adipose mesenchymal stem cells was added to the six-well plate.

(5) Replace fresh human adipose mesenchymal stem cell osteogenic induction differentiation medium every 3 days(preheat to room temperature before use). (**Note: During the process of osteogenic induction, be careful not to hit the liquid to the cell surface when changing the liquid to prevent the cell layer from falling off**.)

(6) After 2 to 4 weeks of induction, the cells should be identified according to the needs of your experiment according to their morphological changes and growth.

3. The use of alizarin red dye solution

When your osteogenic induction experiment is over, alizarin red staining can be used to determine the induction effect(this kit provides alizarin red S staining solution); This process requires preparation of 4% neutral formaldehyde solution and 1×PBS solution.

(1) Absorb the complete osteogenic induction differentiation medium in the orifice plate and rinse it with 1×PBS once or twice.

(2) Add 4% neutral formaldehyde solution(covering the cell surface is enough) and fix the cells for 30min.

(3) Absorb 4% neutral formaldehyde solution and rinse with 1×PBS 1~2 times.

(4) Take the six-hole plate as an example, add 1mL alizarin red dyeing solution to each hole, and stain at room temperature for 30min(dyeing time can be extended or reduced according to the actual situation).

(5) Absorb alizarin red dyeing solution, rinse with 1×PBS 1~2 times, wash the background impurities, you can observe the induction and dyeing effect under the microscope.

Notes:

1. Because the kit has more ingredients, please strictly pay attention to aseptic operation in the preparation process; If you are worried about bad operation in the mixing process, please carry out 0.22μm filter membrane on the complete medium after mixing the reagent to remove bacteria.
2. If stem cells are prone to float or retract during the culture process, the culture plate can be coated with 0.1% gelatin before the induction of stem cells. (Gelatin is not provided in this kit)
3. Dexamethasone B is prepared with anhydrous ethanol and is volatile, take care to tighten the lid.
4. Trace reagent (less than 1mL) must be centrifuged before use.