Rat Adipose Mesenchymal Stem Cells Osteogenic Induction Differentiation Medium

Cat: D3503
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:

Rat adipose mesenchymal stem cells osteogenic induction differentiation medium is specially developed for rat adipose mesenchymal stem cell osteogenic induction differentiation. According to the characteristics of rat adipose mesenchymal stem cells, the formulation of optimized differentiation reagents can increase the osteogenic differentiation effect of rat 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
Rat 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 rat adipose mesenchymal stem cells osteogenic induction differentiation medium

(1) This product is kit type. Before use, each component reagent in the kit should be mixed well.

(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 glycerophosphatase, ascorbic acid and dexamethasone B were added into the induction base medium successively according to the 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: The reagent component in the sterile reagent tube is absorbed, the gun tip is injected below the liquid level of the medium, and the gun tip is slightly blown to wash. 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. Rat adipose mesenchymal stem cells osteogenic induction differentiation operation guidance

This process requires the preparation of rat adipose mesenchymal stem cells complete medium, 0.25% pancreatic enzyme, 1×PBS and rat 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 rat adipose-derived mesenchymal stem cells reaches 80-90%, digestion can be performed with 0.25% pancreatic enzyme.

(2) The digested rat 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 inoculation with the six-well plate**). Each well was inoculated with 2mL of rat adipose mesenchymal stem cells in complete medium.

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

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

(5) Replace the fresh rat adipose mesenchymal stem cell osteogenic induction differentiation medium every 3 days(preheat to above 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 fluid 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. Due to the large number of components in the kit, please strictly pay attention to aseptic operation during 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.