Mouse Adipose Mesenchymal Stem Cells Adipogenic Induction Differentiation Medium

Cat: D3517
Size: 400mL
Storage: The basic medium should be stored in the refrigerator at 4℃, and other components should be stored at -20℃. The validity period is 1 year.

Introduction:

Mouse dipose mesenchymal stem cells adipogenic induction differentiation medium was specially developed for mouse dipose mesenchymal stem cells lipid-induced differentiation. Optimized formulation of differentiation reagents according to the characteristics of mouse dipose mesenchymal stem cells can increase the adipogenic induction differentiation effect of mouse dipose 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:

Adipogenic induction differentiation Medium A solution:

Kit Components	Add volume
Basal Medium For Stem Cells Adipogenic Induction Differentiation A	177mL
Fetal Bovine Serum For Stem Cells Adipogenic Induction Differentiation	20mL
Supplement For mBMSCs Stem Cells Adipogenic Induction Differentiation A①	2.8mL
Supplement For mBMSCs Stem Cells Adipogenic Induction Differentiation A②	200μL

Adipogenic induction differentiation Medium B solution:

Kit Components	Add volume
Basal Medium For Stem Cells Adipogenic Induction Differentiation B	177.2mL
Fetal Bovine Serum For Stem Cells Adipogenic Induction Differentiation	20mL
Supplement For mBMSCs Stem Cells Adipogenic Induction Differentiation B	2.8mL

Auxiliary reagent:

Name	Add volume
Oil Red O Solultion	10mL
Gelatin Solultion	10mL

Note: Each component should be stored according to the temperature indicated on the label on the reagent tube.

Protocols(only for reference):

1. Preparation of adipogenic induction differentiation medium for mouse dipose mesenchymal stem cells

(1) Introduction: This product is kit type. Before use, all components in the kit should be mixed. (Do not confuse solution A with solution B)

(2) Preparation: defrost the serum at 4℃ until it is completely melted. Thaw each additive at room temperature until completely melted, gently shake A①, B mix; A② Short centrifugation, so that all reagents can be collected to the bottom of the tube.

(3) Solution A configuration: FBS(no difference between the two FBS), A①, A② were successively added to the basic medium A; Mix to make a logo, ready to use.

(4) Solution B configuration: FBS (no difference between the two FBS) and B were successively added to the base medium B; Mix to make a logo, ready to use.

Note: In steps 3 and 4, the reagent ingredients in the sterile reagent tube are absorbed, and the gun head is quickly injected under the liquid surface of the medium, and the gun head is gently blown and washed. Then absorb a small amount of medium washing reagent tube, and add all components to the basic medium as completely as possible, which can better ensure the effect of the medium.

2. Mouse dipose mesenchymal stem cells adipogenic induction differentiation operation guidance

Tips:

(1) Reagent preparation: This process requires the preparation of mouse dipose mesenchymal stem cell complete medium, 0.25% pancreatic enzyme, 1×PBS and mouse dipose mesenchymal stem cell adipogenic induction differentiation medium.

(2) Gelatin coating: Gelatin coating helps to reduce cell retraction, flapping, curling, and weak adhesion during cell induction. The operation steps are as follows: "Add an appropriate amount of gelatin coating solution, cover the bottom of the orifice plate, incubate on a super-clean table or cell incubator for 30min, remove the gelatin coating solution, and then it can be used for experimental inoculation".

(3) Temperature: The temperature change of the cell medium is the main factor that causes the flapping and curling in the process of cell induction. Therefore, the induction medium must be preheated to 37℃ before the liquid change, and the time for observing cells outside should not be too long (less than 10 minutes is recommended).

(4) Liquid change: Do not operate too many holes at the same time (recommended within 6 holes), it is recommended to gently and slowly inject along the side wall of the hole plate during liquid change.

This operation guide takes the six-well plate as an example:

(1) When your mouse dipose mesenchymal stem cells have reached 80-90% fusion, they can be digested with 0.25% pancreatic enzyme.

(2) The digested mouse dipose mesenchymal stem cells were counted and inoculated into six-well plates with a cell density of 2-3×10^4cells/cm^2 according to the counting results, and 2mL of mouse dipose mesenchymal stem cells were added into each well.

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

(4) When the degree of cell fusion reached 100%(cell supersaturation was conducive to stimulating the adipogenic-forming potential of stem cells), the complete medium was carefully sucked out of the pores and 2mL mouse dipose mesenchymal stem cells adipogenic induction differentiation medium A solution was added to the six-well plate.

(5) After 2 to 3 days of induction by A solution, the induced complete medium of the six-well plate was sucked away, and 2mL mouse dipose mesenchymal stem cells were added to each well to form adipogenic induction differentiation medium B solution was completely maintained for 1 day.

Note: The induction duration of A solution can be 2-3 days, and the morphological changes of cells during induction are normal. The program of "A2 +B1 days" has a milder stimulation of cells and is more secure for beginners. The "A3 + B1 day" program has a stronger stimulation of cells, and can speed up the experimental process in the case of excellent cell status and experienced operators.

(6) After 3-5 times of induction with medium A and medium B alternately, when obvious and sufficient lipid droplets were observed in stem cells, liquid B could be continued for 3-6 days (liquid change every 2-3 days), until the direct lipid droplets became large and full enough, the induction could be ended, and the cells could be stained and subsequently identified according to the experimental requirements.

3. The use of oil red O dyeing solution

(1) When your adipogenic induction experiment is completed, you can dye oil red O to determine the induction effect (this kit provides saturated oil red O dyeing solution, **which needs to be prepared into working solution before use**).

(2) The adipogenic induction differentiation complete medium in the pore plate was sucked away and washed with 1×PBS 1-2 times.

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

(4) During cell fixation, oil red O working liquid can be prepared (saturated oil red O solution: distilled water =3:2, mixed and filtered with neutral filter paper or nylon material filter membrane to remove impurities).

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

(6) Take the six-hole plate as an example, add 1mL oil red O working liquid to each hole, and dye for 30min at room temperature.

(7) Absorb the oil red O working liquid, rinse with 1×PBS 1-2 times, wash the background impurities, you can observe the induction and staining effect under the microscope.

Notes:

1. Because there are many components in the medium, please pay strict attention to aseptic operation in the preparation process; If you are concerned about bad operation during mixing, please apply 0.22μm filtration membrane to the complete medium after mixing the reagent.
2. The alternating induction of A and B is to reduce the effect of reagents in liquid A on stem cells. If your stem cells are in A good state, you can use only liquid A for stimulation induction in the first 7 days (replace fresh A solution every 2-3 days in the middle), and then perform the alternating induction operation of the two media after the rapid appearance of fat droplets.