Mouse Adipose Mesenchymal Stem Cells Chondrogenic Induction Differentiation Medium

Cat: D3527
Size: 100mL/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:

Mouse adipose mesenchymal stem cells chondrogenic induction differentiation medium was specially developed for mouse adipose mesenchymal stem cells chondrogenic induction differentiation. According to the characteristics of mouse adipose mesenchymal stem cells, the formulation of differentiation reagents was optimized to increase the chondrogenic differentiation effect of mouse 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:

Chondroblast induced differentiation medium:

Kit Components	Add volume -100mL	Add volume-200mL
Basal Medium For Stem Cells Chondrogenic Induction Differentiation	87.6mL	175.2mL
Fetal Bovine Serum For Stem Cells Chondrogenic Induction Differentiation	10mL	20mL
Supplement For mADSCs Stem Cells Chondrogenic Induction Differentiation	2.4mL	2.4mL×2

Auxiliary reagent:

Kit Components	Add volume-100mL	Add volume-200mL
Alcian Blue 8GX Solultion	10mL	10mL×2
Gelatin Solultion	10mL	10mL×2

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 chondrogenic induction differentiation medium for mouse adipose mesenchymal stem cells

(1) Preface: This product is kit type. Before use, it is necessary to mix each component reagent in the kit.
(2) Preparation: defrost the serum at 4℃ until it is completely melted; Defrost the additive at room temperature until completely melted, shake gently and mix well.
(3) Configuration [Serum-additives]: Mix the serum and additives and shake them well. Divide them into several parts and freeze them at -20℃.
(4) Complete configuration: Add 1 volume of [serum-additive] to 7 volume of basic medium, mix well for marking, and then use.

Note: The prepared chondrogenic induction and differentiation medium must be used on the same

day.

2. Mouse adipose mesenchymal stem cells chondrogenic induction differentiation operation guidance

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

(2) The digested mouse adipose mesenchymal stem cells were counted, and according to the counting results, the cells were added to the chondroblast induced complete media at the rate of 7.5× 105 cells/mL, and then centrifuged 150g for 5min;

(3) Discard the supernatant and add it into the chondroblast induced differentiation complete medium(ready for use) at the amount of 5.0× 10^5cells/mL, and then reinsert the cells.

(4) 500μL cell suspension(i.e. 2.5×10^5cells) was absorbed and transferred into a 15mL centrifuge tube, centrifuged 150g for 5min;

(5) Do not shake or blow the cell mass after centrifugation, and carefully loosen the centrifuge tube cover to facilitate gas exchange. Incubate in 37℃, 5% CO_2.

Note: Do not shake the centrifuge tube for 48 hours and keep the centrifuge tube standing.

(6) After 48h, change the cartilage differentiation induction solution every 2 to 3 days, 0.5mL per tube, and flick the cell mass to make it float off the wall.

(7) Unscrew the tube cap and put it in 37℃, 5% CO_2 for further induction; During induction, the diameter of the cell mass will increase and the surface will become smooth and gelatinous.

Note: To make it easier for the cells to coalesce into a ball, choose a centrifuge tube with a rounder bottom.

(8) After 20 to 30 days of continuous induction, the cartilages can be sectored with neutral formaldehyde and paraffin embedding. After secting, staining identification can be performed according to the needs of the experiment. (This kit provides Alcian Blue 8GX Solultion dye solution)

Note: Alcian Blue 8GX Solultion also known as Alcian Blue 8GX Solultion.

3. Alcian Blue 8GX Solultion staining analysis

(1) Fixation: neutral formaldehyde fixation, paraffin embedding section.

(2) Dyeing steps:

1) Dewaxing paraffin sections to water, rinse with distilled water. (Do not rinse the tissue directly, in case the slice is removed and the tissue is damaged);
2) Soak Alcin Blue dye for 30min;
3) Rinse with running water for 2min;
4) distilled water to stop dyeing;
5) Observe the degree of staining under a microscope and take photos.

(3) Interpretation of the result: the cartilage and acidic mucus were blue.

Notes:

1. Because there are more components in the medium, please pay strict attention to aseptic operation in the preparation process; If you are worried about the bad operation in the mixing process, please carry out 0.22μm filter membrane filtration on the complete medium after mixing the reagent.
2. Chondrogenic differentiation additives contain cell growth factors, remember to use now, prepared complete media must be used on the same day.