

## Rat Adipose-derived Mesenchymal Stem Cells Chondrogenic Differentiation Medium

Cat. No. : PD-012

Size : 100mL / 200mL

### Product Description

The Rat Adipose-derived Mesenchymal Stem Cells Chondrogenic Differentiation Medium has been specifically designed to facilitate chondrogenic differentiation. The differentiation reagent's formula has been tailored to the unique characteristics of rat adipose-derived mesenchymal stem cells, thereby enhancing their chondrogenic differentiation potential.

This product is only used for scientific research or further research, not for diagnosis and treatment.

### Component

#### Androgenic Differentiation Medium:

Component name	100 mL	200 mL	Appearance	Storage and Expiration Date
Basal Medium For Stem Cells Chondrogenic Differentiation	87.6 mL	175.2 mL	Red Clear Liquid	2-8°C, 1 year
Nutrient For Stem Cells Chondrogenic Differentiation	10 mL	20 mL	Yellow Clear Liquid	≤-15°C, 5 years
Supplement For Rat Adipose-derived Mesenchymal Stem Cells Chondrogenic Differentiation	2.4 mL	2.4 × 2 mL	Colorless Clear Liquid	-5~-20°C, 1 year

#### Auxiliary Reagents:

Component name	100 mL	200 mL	Appearance	Storage and Expiration Date
Alcian Blue 8GX Solution	10 mL	10 × 2 mL	Blue Liquid	2-8°C, 1 year
Gelatin Solution	10 mL	10 × 2 mL	Colorless Clear Liquid	2-8°C, 1 year

### Preparation of Chondrogenic Differentiation Complete Medium:

1. This product is a kit, please mix the reagents in the sterile reagent bottle before use.
2. Thaw at 4°C and then place to thaw at room temperature until complete, and gently shake during thawing.
3. Mix the Nutrient and Supplement, a [Nutrient-Supplement Mixture] was formed. You can also divide them into several parts frozen at -20°C in order for a long term storage.
4. Add 1 volume [Nutrient-Supplement Mixture] to 7 volumes of Basic Medium, mix it well and mark it, and then it can be used.

**Note:** The complete medium for chondrogenic differentiation should be prepared and used on the same day.

### Guidelines of Chondrogenic differentiation:

1. When your mesenchymal stem cells have reached 80-90% confluence, disperse them with 0.25% trypsin solution.

2. The mesenchymal stem cells suspension was counted to determine the cell concentration.
3. Transfer  $3-4 \times 10^5$  cells into a 15 mL centrifuge tube and centrifuge at 20°C and  $250 \times g$  for 4 minutes.
4. Discard the supernatant liquid. Add 0.5 mL of chondrogenic differentiation induction complete medium to resuspend the cell pellet, and centrifuge at 20°C and  $150 \times g$  for 5 minutes.
5. Repeat step 4 once.
6. After discarding the supernatant, add 0.5 mL of chondrogenic differentiation induction complete medium to resuspend the cells, and centrifuge at 20°C and  $150 \times g$  for 5 minutes.
7. Do not shake or blow the cell mass after centrifugation. Carefully loosen the lid of the centrifuge tube to facilitate gas exchange, and placed in a 37°C incubator with 5% CO<sub>2</sub>.

**Note:** Do not shake the centrifuge tube within 48 hours, keep the centrifuge tube static.

8. After 48 hours, replace the fresh chondrogenic differentiation induction complete medium every 2-3 days, 0.5 mL per tube, and flick the cell mass to float off the wall.
9. Loosen the tube cover and placed in a 37°C incubator with 5% CO<sub>2</sub> to continue induction. During the induction process, the diameter of the cell cluster will increase and the surface will become smooth and glial.

**Note:** In order to make it easier for the cells to gather into a ball, you can choose a more round centrifuge tube at the bottom.

10. After 20-30 days of induction, the cartilage ball can be fixed in neutral formaldehyde and embedded in paraffin. After sectioning, staining can be carried out according to the needs of the experiment. (This kit provides Alcian Blue 8GX Solution).

## Alcian Blue Staining

1. Fixation: neutral formaldehyde fixation, paraffin embedded sections.
2. Staining steps: Dewaxing paraffin sections to water, distilled water rinse. (Do not wash the tissue directly to prevent removal of tablets and destruction of the tissue);
3. Stained with Alcian Blue 8GX Solution for 30 minutes.
4. Rinse with running water for 2 minutes;
5. Distilled water to stop staining;
6. Observe the degree of staining under a microscope and take pictures.
7. Results interpretation: Cartilage and acid mucus were blue.

## Notes

1. This product is only used for scientific research or further research, not for diagnosis and treatment.
2. This product is for scientific research only. It cannot be used for diagnostic, therapeutic, clinical, or other purposes.
3. Please pay attention to aseptic operation during the preparation process. If you are concerned about improper handling during mixing, please filter and sterilize the complete medium with a 0.22 μm filter after mixing the reagents.
4. Chondrogenic differentiation additives contain cell growth factors, please prepared complete medium and used on the same day.