Human Adipose-Derived Mesenchymal Stem Cells Osteogenic Differentiation Medium

Cat. No.: PD-007

Size: 200mL

Product Description

The Human Adipose-Derived Mesenchymal Stem Cells Osteogenic Differentiation Medium is specifically designed to facilitate osteogenic differentiation. The differentiation reagent's formula has been tailored to the unique characteristics of human adipose-derived mesenchymal stem cells, thereby enhancing their osteogenic differentiation potential.

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

Component

Osteogenic Differentiation Medium：

Component name	Volume	Appearance	Storage and Expiration Date
Basal Medium For Stem Cells Osteogenic Differentiation	177 mL	Red Clear Liquid	2-8℃, 1 year
Nutrient For Stem Cells Osteogenic Differentiation	20 mL	Yellow Clear Liquid	≤-15℃, 5 years
Supplement For Human Adipose-Derived Mesenchymal Stem Cells Osteogenic Differentiation	3 mL	Colorless Clear Liquid	-5~-20℃, 1 year

Auxiliary Reagents：

Component name	Volume	Appearance	Storage and Expiration Date
Alizarin Red S (pH=4.1-4.3)	10 mL	Red Liquid	2-8℃, 1 year
Gelatin Solution	10 mL	Colorless Clear Liquid	2-8℃, 1 year

Preparation of Osteogenic Differentiation Complete Medium:

1. All Differentiation Medium Reagents need to be mixed evenly, before using.
2. Preparation: Thaw the "Nutrient For Stem Cells Osteogenic Differentiation" at 4°C until completely melted, the day before use. Thaw the "Supplement For Stem Cells Osteogenic Differentiation" at room temperature until completely melted, and then gently shake and mix it well.
3. Complete medium configuration: Add the "Nutrient For Stem Cells Osteogenic Differentiation" and "Supplement For Stem Cells Osteogenic Differentiation" to the "Basal Medium For Stem Cells Osteogenic Differentiation", and then the "Osteogenic Differentiation Complete Medium" could be used.

   Important Notes:

   1) The prepared complete medium should be stored at 4°C in the dark and used up within 1 month.
   2) If all the culture medium cannot be used up in a short period, it is recommended to prepare it in batches. Firstly, divide the reagents according to the proportion of each component in the

kit, it is recommended to divide no more than 4 portions. When using, take out one of them and prepare the culture medium in proportion, and the remaining components should be stored strictly according to their respective conditions and should not be frozen and thawed multiple times.

Guidelines of Osteogenic differentiation:

Warm Tips：

1. Experimental Reagent List: Complete Medium for normal culture of mesenchymal stem cells, 0.25% Trypsin (contains EDTA), PBS, deionized water or ultrapure water, and Human Adipose-Derived Mesenchymal Stem Cells Osteogenic Differentiation Medium.
2. Gelatin coating: Gelatin coating helps to reduce the phenomenon of cell retraction, floating, curling, non-adhesion and so on in the process of cell induction. You can choose whether to coat the culture vessel according to your cell status. The coating operation steps are as follows: "aseptic operation, add appropriate amount of gelatin coating solution, cover the bottom of the well plate, incubate in an ultra-clean table or cell culture incubator for 30 minutes, and suck out the gelatin coating solution, then the gelatin-coated plate can be used for experimental inoculation".
3. Temperature: In the process of osteogenic induction, the cells are in the state of monolayer membrane at the bottom of the culture plate, which will shrink when cold, resulting in the failure of the experiment. Therefore, the low temperature of medium is one of the main factors that lead to the cell floating and curling during the induction process.
4. Replacing medium: The process of “Replacing medium" is extremely important for osteogenic induction experiments. The valuable experience is summarized as follows for reference:
    1) Before replacing medium, it is recommended to preheat a certain amount of Complete Osteogenic Differentiation Medium required for this operation to 37°C in an incubator or water bath.
    2) The time for “observing cells under a microscope" or “replacing medium "should not be too long (it is recommended to control within 10 minutes, and batch processing is recommended when there are many cells to be processed).
    3) During the “Replacing medium" process, it is recommended to place an empty culture plate (or foam plate) under the culture plate to separate the cold ultra-clean table to prevent the cells from losing temperature rapidly.
    4) After the culture plate is laid flat on the empty culture plate, the plate should be placed flat, and about 80% of the old medium is directly sucked out (it is not recommended to suck out all the old medium, and about 20% of the old medium can be kept moist at the bottom and early trace calcium deposits can be retained).
    5) Slowly inject fresh osteogenic differentiation complete medium along the wall of the well. Be careful not to blow the liquid directly at the cell surface to prevent the cell layer from falling off. After “Replacing medium", gently put it into a 37°C incubator to continue the culture.
    6) Note: If here is many wells need to be replaced medium in a plate (such as 12 and 24 wells cell culture plate), it is not recommended to replace the medium for all the wells at the same time (Don't suck out all the wells at the same time and replace with fresh induction medium at the same time). It is recommended to operate well by well or operate part of the wells (such as

within 6 wells) at the same time.

Example of guidance for osteogenic induction operation (Six-Well Plate):

1. When the fusion degree of human adipose-derived mesenchymal stem cells reaches 80%-95%, add some 0.25% Trypsin (contains EDTA) to disperse the cells.
2. Count the dispersed cells. According to the counting results, seed them in a six-well plate at a density of 2-3 × 10^4 cells/cm^2 (according to the cell's growth rate, adjust the cell seeding quantity to make the confluence degree of mesenchymal stem cells reached 80%-95% after seeding cells for 24-72 hours). Add 2 mL of human adipose-derived mesenchymal stem cell complete medium to each well.
3. The human adipose-derived mesenchymal stem cells inoculated evenly were cultured in an incubator at 37°C and 5% CO_2.
4. When the cell fusion degree reaches 80%-95%, carefully discard the complete medium in the well, and then add 2 mL Osteogenic Differentiation Complete Medium of human adipose-derived mesenchymal stem cells to each six-well plate.
5. Replace fresh preheated Osteogenic Differentiation Complete Medium every 48-72 hours
6. After 2 to 4 weeks of induction, pay attention to the morphological changes and growth of the cells, and identify them according to the needs of the experiment.

Alizarin Red Staining to detect Differentiation Effect (Six-Well Plate)

After the osteogenic induction experiment, alizarin red staining can be performed to determine the induction effect (alizarin red S staining solution is attached to this product). You need to prepare 4% paraformaldehyde solution, PBS and deionized water or ultrapure water solution by yourself.

1. Throw away the osteogenic differentiation complete medium in the well and wash it with PBS for 1-2 times.
2. Add 4% paraformaldehyde solution (cover the cell surface) and fix the cells for 10 minutes.
3. Throw away the 4% paraformaldehyde solution and wash it with deionized water or ultrapure water for 1-2 times.
4. Take six-well plate as an example, add 1 mL alizarin red staining solution to each well and dye for 5-10 minutes at room temperature (the staining time can be extended or reduced according to the actual situation).
5. Throw away the alizarin red staining solution, wash it with deionized water or ultrapure water 1-2 times (wash the background impurities), then observe the induction and staining effects under a microscope (keep a small amount of deionized water or ultrapure water for photographing).
6. Results Interpretation: Calcium nodules are red, red to yellow, or red to purple after alizarin red staining.

Notes

1. This product is only used for scientific research or further research, not for diagnosis and treatment.
2. Due to the long time of osteogenic differentiation experiment, please pay strict attention to aseptic operation during the preparation and use of this product.
3. During the osteogenic induction experiment, it is difficult to determine whether the induction has been completed by microscopic observation or cell photos, and the plate can not continue to be

induced once stained. Therefore, it is recommended that "at the beginning of the experiment, more (1-3) parallel experiments should be carried out on another well plate at the same time, or only the 'induction control group' should be carried out on another well plate (1-3) more, to judge the induction progress of formal experiments by alizarin red staining at the later stage of induction".

4. The color of “Supplement For Stem Cells Osteogenic Differentiation" is white to yellow, and does not affect normal use.
5. After melting, the “Supplement For Stem Cells Osteogenic Differentiation” has a slight white precipitate, which is L-glutamine. The osteogenic differentiation supplement can be directly added to the “Basal Medium” without affecting normal use.

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