3T3-L1 (Mouse embryonic fibroblasts) Adipogenic Differentiation Medium

Cat.No. : PD-031

Size: 400mL

Product Description

The 3T3-L1 (Mouse embryonic fibroblasts) Adipogenic Differentiation Medium has been specifically designed to facilitate adipogenic differentiation. The differentiation reagent's formula has been tailored to the unique characteristics of 3T3-L1 (Mouse embryonic fibroblasts), thereby enhancing their adipogenic differentiation potential.

This product is exclusively intended for scientific research. It must not be used for diagnostic, therapeutic, clinical, or other purposes.

Component

Adipogenic Differentiation Medium A:

Component name	Volume
Basal Medium For Stem Cells Adipogenic Differentiation A	177 mL
Nutrient For Stem Cells Adipogenic Differentiation	20 mL
Supplement For 3T3-L1 (Mouse embryonic fibroblasts) Adipogenic Differentiation A①	2.8 mL
Supplement For 3T3-L1 (Mouse embryonic fibroblasts) Adipogenic Differentiation A②	200 µL

Adipogenic Differentiation Medium B:

Component name	Volume
Basal Medium For Stem Cells Adipogenic Differentiation B	177.2 mL
Nutrient For Stem Cells Adipogenic Differentiation	20 mL
Supplement For 3T3-L1 (Mouse embryonic fibroblasts) Adipogenic Differentiation B	2.8 mL

Auxiliary Reagents:

Component name	Volume
Oil Red O Solution	10 mL
Gelatin Solution	10 mL

Preparation of Adipogenic Differentiation Complete Medium

1. All Differentiation Medium Reagents need to be mixed evenly before using. Do not confuse ‘Medium A’ with ‘Medium B’.
2. Preparation: Thaw the ‘Nutrient For Stem Cells Adipogenic Differentiation’ at 4℃ until completely melted, the day before using. On the day of use, thaw all the ‘Supplement For Stem Cells Adipogenic Differentiation’ at room temperature until completely melted, and then, gently shake ‘A①’ & ‘B’ and make itself mixed well. All A② reagents should be collected at the bottom of the tube by briefly centrifugation.

3. 'Adipogenic Differentiation Medium A' Preparation: In sequence add the 'Nutrient For Stem Cells Adipogenic Differentiation', 'Supplement A①' and 'Supplement A②' to the basic medium A. Mix well, mark and then the 'Adipogenic Differentiation Medium A' could be used.
4. 'Adipogenic Differentiation Medium B' Preparation: In sequence, add the 'Nutrient For Stem Cells Adipogenic Differentiation', 'Supplement B' to the basic medium B. Mix well, mark and then the 'Adipogenic Differentiation Medium B' could be used.
5. Note: About 'Supplement A②', should thaw it at normal temperature (25-37℃), use dry and sterile 200 μL pipet tips to gently mix well, Suck 'Supplement A②', so that the pipet tip is hovering over the liquid level and quickly injected into the basic medium A . The prepared complete culture medium shall be stored at 4℃ in the dark and shall be used up within 1 month.
6. Note: When handling 'Supplement A②', ensure that you use a new, dry pipette tip to transfer 'Supplement A②' into the base medium. Avoid adding the base medium to 'Supplement A②', as this may lead to precipitation.

Guidelines of Adipogenic differentiation:

Warm Tips:

1. Experimental Reagent List: Prepare '3T3-L1 (Mouse embryonic fibroblasts) Cell Complete Medium', 0.25% trypsin, 1× PBS and '3T3-L1(Mouse embryonic fibroblasts) Adipogenic Differentiation Medium (PD-031)'.
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 the 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 discard out the gelatin coating solution, then the gelatin-coated plate can be used for experimental inoculation.
3. Temperature: In the process of adipogenic induction, the cells are in the state of monolayer membrane at the bottom of the culture plate, which will shrink when cold, failing 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. Replace medium: The process of 'Replace medium' is extremely important for adipogenic induction experiments. The valuable experience is summarized as follows for reference:
    1) Before replacing the medium, it is recommended to preheat a certain amount of Complete Adipogenic 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 discarded out (it is not recommended to discard out all the old medium, and about 20% of the old medium can be kept moist at the bottom).
5) Slowly inject fresh adipogenic 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℃ incubator to continue the culture.
6) Note: If 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 discard 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.

Adipogenic induction operation guide using a six-well plate as an example

1. When the confluence degree of mesenchymal stem cells reaches 80%-90%, add some 0.25% Trypsin (Contains EDTA) to disperse the cells.
2. Count the dispersed cells. According to the counting results, seed the cells at a density of 2-3 × 10^4 cells/cm^2 to a six-wells plate (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 to 72 hours). Add 2 mL of Complete Growth Medium to each well.
3. The evenly seeded mesenchymal stem cells were cultured in an incubator at 37℃ and 5% CO_2.
4. When the cell confluence degree reached 80%-95%, carefully discard the old Complete Growth Medium in the well, and then add 2 mL 'Adipogenic Differentiation Medium A' into each well.
5. After 2-3 days of induction with 'Adipogenic Differentiation Medium A', remove the 'medium A' from the six-well plate, and 2 mL of 'Adipogenic Differentiation Medium B' is added to each wells.
6. After 1 days of induction with 'Adipogenic Differentiation Medium B', remove the 'medium B' from the six-well plate, and 2 mL of 'Adipogenic Differentiation Medium A' is added to each wells.
7. Note: The induction time of 'Adipogenic Differentiation Medium A' can be 2-3 days, and the morphological changes of cells during induction are normal. The program of "A for 2 days & B for 1 day" has milder stimulation to cells and is safer for beginners. The program of "A for 3 days & B for 1 day" has stronger stimulation to cells and can speed up the experimental process under the condition of excellent cell condition and rich operator experience.
8. After 3-5 times of alternating induction with 'medium A' and 'medium B', when obvious and sufficient lipid droplets are observed in stem cells, only using 'medium B' continue to culture for 3-6 days (every 2-3 days to change the liquid once), until the lipid droplets become large enough, it can be finished.

Oil Red O staining

Detection of Adipogenic differentiation result (example as 6-wells plate):

1. After your adipogenic induction experiment is completed, you can perform Oil Red O staining to test the induction result (this kit provides saturated Oil Red O, and it should be prepared into a working solution before use).

2. Remove the ‘adipogenic induction differentiation medium’ from the wells and rinse 1-2 times with 1× PBS.
3. Add 4% paraformaldehyde solution (cover the cell surface) and fix the cells for 30 minutes.
4. During cell fixation, oil red O working solution can be prepared (saturated oil red O solution: distilled water = 3:2, mixed well and filtered with neutral filter paper or nylon filter membrane to remove impurities).
5. Remove 4% paraformaldehyde solution and rinse 1-2 times with 1× PBS.
6. Taking a 6-well plate as an example, add 1mL of Oil Red O working solution to each well and dye at room temperature for 30 minutes.
7. Remove the Oil Red O working solution, rinse 1-2 times with 1× PBS, clean the background impurities, and observe the induction and staining effects under a microscope.

Notes

1. Due to the long duration of the Adipogenic differentiation experiment, please strictly pay attention to the aseptic operation during the use of our product.
2. The alternating induction of A and B is to reduce the bad influence of ‘medium A’ on stem cells. When your stem cells are in very good condition, you can use only ‘medium A’ in the first 7 days (fresh medium A is replaced every 2-3 days), after lipid droplets appear rapidly, continue the induction differentiation process by the alternation of ‘medium A’ & ‘medium B’.