

## Product Information

This product is an adipogenesis differentiation medium kit Balb/c BMSCs into adipocytes. All products in the kit have passed biosafety testing and product quality testing with stable and effective performance.

## Basic Information

Components	Specification	Storage	Validity
Differentiation Medium (Contains Glutamax, Penicillin-Streptomycin and Inducible Factors)	100 mL	2~8°C, protected from light	3 months
Maintenance Medium (Contains Glutamax, Penicillin-Streptomycin and Inducible Factors)	100 mL	2~8°C, protected from light	3 months
Dye Liquor: Oil Red O Solution	5 mL	2~8°C, protected from light	12 months

## Quality inspection standards

pH: 7.2~7.4

Endotoxin content: <10 EU/mL

Biosafety: Negative for bacteria, fungi, and mycoplasma

Purpose: Enhancing the ability of Balb/c BMSCs to induce differentiation into adipocytes

## Principle

Oil Red O dye, a member of the Sudan dye family, is a lipid-soluble azo dye. It has obvious color development and is easy to observe. It is mainly used for fat staining. Under the action of the induction culture medium, stem cells will gradually differentiate into pre-adipocytes and adipocytes, and will form lipid droplets of different sizes. The solubility of Oil Red O in fat is greater than its solubility in the staining solution, thereby coloring the fat red or orange-red.

## Operation steps

### 1. Adipogenic differentiation procedure

#### 1.1 Inoculation of stem cells

Take cells in the logarithmic growth phase, inoculate them into culture vessels at a cell density of  $2.0 \times 10^4$  cells/cm<sup>2</sup>, and culture them at 37°C, 5% CO<sub>2</sub> until the confluence is 90-100%, discard the supernatant, and add adipogenic differentiation medium.

**Note: If the cells have poor adhesion, it is recommended to use 0.1% gelatin to coat the culture bottom surface.**

#### 1.2 Cell differentiation induction

At 37°C, 5% CO<sub>2</sub> to culture cells about 3 days, then replace with adipogenic maintenance medium. After 1 day of culture, replace with adipogenic differentiation medium again and continue culturing cells for 3 days. Induce for 14-21 days according to the above medium change frequency, and pay attention to observe the changes in cell morphology. According to the number and size of lipid droplets formed by cell induction, decide when to terminate cell induction, and perform staining identification.

### 2. Staining identification

#### 2.1 Cell fixation

Aspirate the culture medium and wash once with an appropriate amount of 1×PBS. Discard it and take an appropriate amount of 4% neutral formaldehyde solution to cover the bottom of the culture dish. Fix it at room

temperature for 30-60 min. Discard the fixative and wash twice with 1×PBS.

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#### 2.2 Oil Red O staining

Prepare Oil Red O working solution with physiological saline or 1×PBS and Oil Red O stock solution (Oil Red O stock solution: physiological saline = 3:2), and prepare it before use. After preparation, the Oil Red O working solution can be centrifuged to precipitate the supersaturated precipitate in the staining solution. Add an appropriate amount of Oil Red O working solution to the cleaned induction wells and let it stand for staining for 30 minutes. Aspirate the Oil Red O working solution, wash twice with 1×PBS, and add an appropriate amount of 1×PBS to prevent the cells from drying out.

#### 2.3 Induction evaluation

Observe the adipogenic staining effect under a microscope, and perform image acquisition and induction evaluation. When the induction is successful, the lipid droplets will appear red or orange after combining with Oil Red O dye.

**Note: The adipogenic differentiation level of stem cells varies depending on factors such as cell type, cell donor source, culture conditions, cell passage, cell state and differentiation time.**

#### Notes

1. The components of this product are all sterile and can be directly prepared into complete culture medium; the staining solution is an independently packaged component and should not be mixed with the culture medium
2. Before preparing complete culture medium, please centrifuge each tube of small-dose reagent to avoid loss. After preparation, please use it within the validity period.
3. Storage conditions of complete culture medium: 2~8 °C , protected from light; the shelf life is 3 months after preparation .
4. This product is For Research Use Only, Not for Diagnostic Use.