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# Alpha-MEM, Nucleoside-Free (with Glucose, Glutamine, Phenol Red, Sodium Pyruvate, Earle's Salts) Product manual

## Basic Information

Cat.NO	Size	Shelf	Form	Storage	Transportation
CMB0038	2L	24 months	Powder	Store at 2-8°C away from light	Room temperature

## Product Introduction

MEM medium (Minimum Essential Medium) is also called the minimum essential medium, minimum basic medium or low-limit Eagle medium. It was developed by Harry Eagle based on Eagle Basic Medium (BEM). It is the most basic and widely applicable medium and one of the most commonly used mediums in animal cell culture. MEM medium contains only 12 essential amino acids, glutamine and 8 vitamins. It has simple ingredients and is mainly used for the culture of adherent cells. It can also be used for other types of cell culture after the formula is modified. MEM medium containing NEAA (non-essential amino acids) is based on MEM medium and adds 7 NEAAs: L-alanine, L-glutamic acid, L-asparagine, L-aspartic acid, L-proline, L-serine and glycine. It can reduce the side effects of the production of non-essential amino acids by cells themselves during cell culture and effectively promote cell proliferation and metabolism.

Earle's Balanced Salt Solution (EBSS) is one of the most commonly used phosphate buffers. Its main components include NaCl, KCl, Na<sub>2</sub>HPO<sub>4</sub>, NaHCO<sub>3</sub>, etc. It has the functions of maintaining osmotic pressure, keeping pH stable, and providing simple nutrition.

### Preparation of powder

1. Purified water, ultrapure water or water for injection should be used for preparation, and the water temperature should be controlled at 20-30°C during the preparation process;
2. Add 90% of the preparation volume of preparation water to the preparation container (if 1L is required, add 900mL of preparation water here), turn on the mixing system of the culture medium preparation container (it is recommended that the input power per unit

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volume of the mixing system is greater than  $10W/m^3$ ), stir well, and avoid the generation of bubbles during stirring;

3. Weigh the powder culture medium according to the required preparation volume. Add the accurately weighed culture medium powder to the preparation container of step 2 and stir thoroughly for more than 20 minutes until the powder is completely dissolved;
4. Add preparation water to accurately make the completely dissolved solution of step 3 to 100% of the preparation volume (if 1L is required, make it to 1L);
5. Measure the pH value and adjust the pH value to 7.20-7.30 with 1mol/L sodium hydroxide solution or 1mol/L hydrochloric acid solution if necessary; since filtration will slightly increase the pH value of the culture medium, it should be lower than the target pH value (7.20-7.40);
6. Sterilize by positive pressure filtration with a filter membrane with a pore size of  $0.2\mu m$  (pay attention to aseptic operation);
7. After filtration, take a small amount of liquid culture medium for bacterial inspection and use it after it passes the test;
8. The filtered culture medium liquid should be used immediately or stored in a glass bottle, culture medium bottle (PET) or disposable liquid storage bag with oxygen barrier coating, and stored at  $2-8^{\circ}C$  away from light. At this time, the shelf life of the liquid culture medium is 1 year.

## Instructions

1. Balance the culture medium and related solutions in a water bath or at room temperature, and prepare the culture medium required for the experimental cells;
2. Cell inoculation: Remove the cells to be cultured from the original culture container, wash with appropriate culture medium or PBS, and adherent cells need to be digested with trypsin;
3. Collect the cells by centrifugation, centrifuge at 1000rpm for 3 min at room temperature, and discard the supernatant;
4. Add fresh culture medium to resuspend the cells. Then inoculate the cell suspension into the culture bottle with the corresponding volume of culture medium, mix gently, and culture at  $37^{\circ}C$  and 5%  $CO_2$  saturated humidity. Observe and replace fresh culture medium regularly according to cell growth and cell density.

## Precautions

1. During the entire process, be sure to pay attention to aseptic operation to avoid contamination;
2. To maintain the best use effect of this product, do not perform freeze-thaw treatment;
3. This product is only used for research or further research, not for diagnosis and treatment.