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# DMEM high glucose (without HEPES, sodium pyruvate, phenol red, glutamine) Product manual

## Basic Information

Cat.NO	Size	Shelf	Form	Storage	Transportation
CMB0002	2L	24 months	Powder	Store at 2-8°C away from light	Room temperature

## Product Introduction

Dulbecco's Modified Eagle Medium (DMEM) is developed on the basis of MEM medium. Compared with MEM medium, the amino acid content is increased by 2 times, the vitamin content is increased by 4 times, and non-essential amino acids, trace iron ions and sodium pyruvate are also added. The glucose content of DMEM medium was originally designed to be 1000 mg/L (low glucose type), and later it was developed to have a glucose content of 4500 mg/L (high glucose type). It is now widely used in the culture of various cells. This product contains a variety of ingredients such as amino acids, vitamins, inorganic salts, etc. required for the culture of various types of cells, but does not contain proteins, lipids or any growth factors, so this product must be used with serum or serum-free additives.

### 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 volume of the mixing system is greater than 10W/m<sup>3</sup>), 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, stir thoroughly for more than 20 minutes until the powder is completely dissolved;
4. After the solution is completely clarified, weigh sodium bicarbonate (analytical grade)

- powder at a ratio of 3.7g/L according to the preparation volume, slowly add it to the solution of step 3, and continue stirring for 5-10 minutes until dissolved;
5. Add preparation water to accurately make the completely dissolved solution 100% of the preparation volume (if 1L is required, make it 1L);
  6. 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; because filtration will make the pH value of the culture medium slightly higher, it is lower than the target pH value (7.20-7.40);
  7. Use a filter membrane with a pore size of 0.2 $\mu$ m to sterilize by positive pressure filtration (pay attention to aseptic operation);
  8. After filtration, take a small amount of liquid culture medium for bacterial inspection and use it after it passes the test;
  9. The filtered culture medium liquid should be used immediately or stored in glass bottles, culture medium bottles (PET) or disposable storage bags with oxygen barrier coatings and stored at 2-8°C away from light. The shelf life of the liquid culture medium is 1 year.

Phenol red is used as a pH indicator in culture media to continuously monitor the pH of the culture medium. At low pH values, phenol red makes the culture medium yellow, while at higher pH values, the culture medium turns purple. It turns red at pH 7.2-7.4, which is most suitable for cell culture. However, phenol red also has some disadvantages. Studies have shown that phenol red can simulate the effects of steroid hormones (especially estrogen). Therefore, when using estrogen-sensitive cells (such as breast tissue), it is best to use a culture medium that does not contain phenol red. Phenol red can interfere with detection during flow cytometry analysis. In addition, the presence of phenol red in some serum-free culture medium formulas can interfere with sodium-potassium balance.

L-glutamine is a necessary nutrient element in cell culture, but it is unstable in solution and will degrade spontaneously. The medium without L-glutamine can adjust the amount of L-glutamine according to research needs. Adding fresh L-glutamine or its substitute during use is more conducive to cell growth.

## 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°C and 5% CO<sub>2</sub> 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.