M199 (with glucose, glutamine, Earle's salts) Product manual

Basic Information

Cat.NO	Size	Shelf	Form	Storage	Transportation
CMB0028	2L	24 months	Powder	Store at 2-8°C away from light	Room temperature

Product Introduction

M199 stands for Medium 199, which was designed by Morgan et al. in 1950. It was originally used for nutritional research on chicken embryo fibroblasts and is now widely used in the culture of various animal cells, including some non-mammalian animal cells. M199 is particularly suitable for the culture of non-transformed cells. It is also commonly used in virology, vaccine production, and the culture of rat pancreatic epithelial cells and mouse lens tissue. Compared with other basal culture media, M199 contains unique ingredients, including adenine, adenosine, hypoxanthine, thymine, and other vitamins. M199 has two balanced salt components. Earle's salt components are often used in CO2 environments, and Hank's salt components are used in non-CO2 environments. This product contains a variety of ingredients such as amino acids, vitamins, inorganic salts, etc. required for various types of cell culture, but does not contain proteins, lipids or any growth factors. Therefore, this product needs to be used with serum or serum-free supplements.

Earle's Balanced Salt Solution (EBSS) is one of the most commonly used phosphate buffers. Its main components include NaCl, KCl, Na2HPO4, NaHCO3, 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℃ 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/m³), 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 0.2μm (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℃ 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°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.