# Instructions for PEG Phosphate Solution

**Cat:** Store at 2-8℃, 6 months.

## Introduction

PEG phosphate solution is mainly composed of PEG, phosphate, etc. Its principle of action is to change the membrane structure of various types of cells, causing the lipid molecules on the plasma membrane at the contact point of two cells to disperse and recombine. The interface between two cells is located at the bilayer plasma membrane, and under the mutual affinity and surface tension of each other, cells fuse. PEG phosphate solution can be used as a melting agent to obtain hybridoma cells for producing monoclonal antibodies and induce cell hybridization. The reagent undergoes strict aseptic treatment.

| Cat No. | Product |
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| IP9310 | PEG8000 Phosphate Solution (50%, Sterile) |
| IP9311 | PEG4000 Phosphate Solution (50%, Sterile) |
| IP9312 | PEG3350 Phosphate Solution (50%, Sterile) |
| IP9313 | PEG1000 Phosphate Solution (50%, Sterile) |
| IP9314 | PEG1500 Phosphate Solution (50%, Sterile) |
| IP9315 | PEG1450-DPBS Solution (50%, Sterile) |

## Protocols (*only for reference*)

***Self-prepared materials: MEM medium, Fetal bovine serum, HAT, HT, A Media Supplement, Trypsin digestive fluid**

1. If it becomes jelly-like, it can be turned into a solution in a water bath of 37~60℃.
2. Single layer adherent cells: Inoculate the precursor cells of hybridization in the same quantity (5 × $10^4$/mL), culture the cells in appropriate medium, and wait for the cells to adhere and expand to the density of the confluence sheet (80% confluence rate). Aspirate the culture medium, add 2 mL of PEG phosphate solution, gently rotate for 1 min to cover all cells with PEG phosphate solution, let it stand for 1 min, add 5 mL of complete MEM culture medium to dilute PEG phosphate solution, remove the diluted PEG phosphate solution, wash the cells treated with PEG phosphate solution with 5 mL of MEM culture medium, remove the washing solution, add 5 mL of MEM culture medium, culture overnight at 37℃ and 5% $CO_2$; After 24-48 h, first aspirate the culture medium and add trypsin digestion solution to treat the cells.After the cells are digested, aspirate the trypsin digestion solution and use HAT to selectively culture and remove HPRT and TK deficient cells; Discard the supernatant and resuspend the cells in complete culture medium supplemented with 1 × HT and 1 × A. After fusion for 12-24 h, perform heterokaryotic analysis. The hybrid precursor cells die within 4-5 days, and for most fusion precursor cells, hybrid cell clones can be seen within 10-14 days.
3. Suspension cells:1 mL of cells from each of the two different parental bodies (approximately 1 × $10^7$) was mixed, centrifuge at 800 xg for 10 min to precipitate the hybrid precursor cells, discard the

supernatant to leave about 1mL, gently tap the bottom of the tube or hand crank the centrifuge tube to mix and resuspend the two cells, add 1mL of PEG phosphate solution to the centrifuge tube, place in a 37℃ water bath for 2 min, add 5 mL of MEM culture medium containing 10% fetal bovine serum preheated at 37℃ in advance, dilute and stop the PEG phosphate solution, centrifuge at 1000 xg for 5 min, discard the supernatant, add 5 mL of complete MEM culture medium to dilute the PEG phosphate solution, and aspirate the diluted PEG phosphate solution.Using 5 mL serum-free MEM culture medium, resuspend cells by hand centrifuge tube (do not destroy cells), centrifuge at 1000 xg for 5 min, discard the supernatant, repeat this step once, add HAT selective culture medium containing 20% fetal bovine serum, mix well, dilute the cell suspension with culture medium to $5 \times 10^4$/mL, transfer to a 96 well plate (0.1 mL per well) or other vessel, incubate overnight at 37℃ and 5% $CO_2$ for 24-48 h, and select fusion cells.

**Note**

1. For your safety and health, please wear a laboratory coat and use disposable gloves when operating.
2. Please use the reagent as soon as possible after opening to prevent the effect of subsequent experiments.
3. The reagent is only used in the field of scientific research and is not suitable for clinical diagnosis or other purposes.
4. When washing cells in the process of cell culture, attention should be paid to aseptic operation to avoid being contaminated by microorganisms.
5. Both monolayer adherent cells and suspension cells cultured in vitro can be fused, but the success probability is higher for monolayer adherent cells.
6. If you need customised products, please contact us.