V02

PEG8000 溶液(50%，无菌)

货号：G0563
规格：10mL/100mL
保存：2-8℃保存，有效期 6 个月。

产品介绍：

聚乙二醇(Polyethylene glycol，PEG)有众多品种，常见的有 PEG400、PEG1500、PEG4000、PEG6000、PEG8000 等，聚乙二醇系列产品无毒、无刺激性，味微苦，具有良好的水溶性，并与许多有机物组份有良好的相溶性，具有优良的润滑性、保湿性、分散性、粘接剂、抗静电剂及柔软剂等，在化妆品、制药、化纤、橡胶、塑料、造纸、油漆、电镀、农药、金属加工及食品加工等行业中均有着极为广泛的应用。依相对分子质量不同而性质不同，从无色无臭黏稠液体至蜡状固体，分子量 200～600 者常温下是液体，分子量在 600 以上者就逐渐变为半固体状，随着平均分子量的不同，性质也有差异，从无色无臭粘稠液体至蜡状固体，随着分子量的增大，其吸湿能力相应降低；其中 PEG4000 和 PEG6000 常用于促进细胞融合或原生质体融合并有助于生物体(如酵母菌)在转化中摄入 DNA。

PEG8000 溶液(50%, 无菌)主要由 PEG8000 和磷酸盐等组成，经过严格无菌处理。其作用原理是能够改变各类细胞的膜结构，使两细胞接触点处质膜的脂类分子发生疏散和重组，两细胞接口处在双分子层质膜的相互亲和以及彼此的表面张力作用下，细胞发生融合。PEG8000 溶液(50%, 无菌)可用做融合剂，以获得生产单克隆抗体的杂交瘤细胞。该试剂仅用于科研领域，不适用于临床诊断或其他用途。

自备材料:

1. CO2 培养箱、离心机
2. MEM 培养基、胎牛血清、HAT、HT、A Media Supplement、胰蛋白酶消化液

操作步骤：*(仅供参考)*

注意：该液体如果变成胶冻状，可置于 37~60℃水浴加温使其变成溶液。

对于单层贴壁细胞:

1. 将杂交前体细胞以相同数量(5x10^4/ml)接种，以适当的培养基培养细胞，待细胞贴壁扩展至汇合成片(80%汇合率)的密度。
2. 吸干培养液，加入 2ml PEG8000 溶液(50%,无菌)，轻轻转动 1min 使 PEG8000 溶液覆盖所有细胞，静置 1min。
3. 加入 3ml 完全 MEM 培养液以稀释 PEG8000 溶液，吸干净稀释的 PEG8000 溶液，再加入 5ml MEM 培养液洗涤一次被 PEG8000 处理的细胞。
4. 吸干净洗液，再加入 5ml MEM 培养液，37℃ 5%CO2 条件培养过夜。
5. 培养 24~48h 后先吸去培养液，然后加入胰酶消化液处理细胞，待充分消化后吸弃胰酶消化液。
6. 用 HAT 选择培养剔除 HPRT 和 TK 缺陷细胞，弃上清液，用补加 1×HT 和 1×A 的完全培养液重新悬浮细胞，融合 12~24h 后进行异核体分析，杂交前体细胞在 4~5 天内发生死亡，对于大多数融合前体细胞而言，10~14 天可见杂交细胞克隆。

对于悬浮细胞：

1. 将两种不同亲体的细胞各 1ml(约为 1×10^7)混匀，800g 离心 10min 以沉淀杂交前体细胞，弃上清液，使之剩余约 1ml，手指轻弹管底或手摇离心管使两种细胞混匀并重新悬浮。
2. 在离心管中加入 1ml PEG8000 溶液(50%,无菌)，置于 37℃水浴 2min。
3. 加入 5ml 提前 37℃预热的含 10%胎牛血清的 MEM 培养液，使 PEG8000 稀释并停止作用，1000g 离心 5min，弃上清液，加入 5ml 完全 MEM 培养液以稀释 PEG8000 溶液，吸干净稀释的 PEG8000 溶液，加入 5ml 无血清 MEM 培养液，手摇离心管重悬细胞(不要破坏细胞)，1000g 离心 5min，弃上清液，重复 1 次该步骤。
4. 加入含有 20%的胎牛血清的 HAT 选择培养液，混匀，将细胞悬液用培养液稀释至 5×10^4/ml，接种于 96 孔板(每孔 0.1ml)或其他器皿中，37℃5%CO2 孵育过夜，24~48h 后选出融合细胞。

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注意事项：

1. 应注意无菌操作，避免被微生物污染。
2. 体外培养的单层贴壁细胞或悬浮细胞均可做融合，但成功概率较大的是单层贴壁细胞。
3. 试剂开封后请尽快使用，以防影响后续实验效果。
4. 为了您的安全和健康,请穿实验服并戴一次性手套操作。

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PEG8000 Solution(50%, Sterilized)

Cat: G0563
Size: 10mL/100mL
Storage: 2-8℃, valid for 6 months.

Introduction

Polyethylene glycol (PEG) has many varieties, including PEG400, PEG1500, PEG4000, PEG6000, PEG8000, etc. The PEG series products are non-toxic, non irritating, slightly bitter in taste, and have good water solubility. They also have good compatibility with many organic components, and have excellent lubricity, moisturizing, dispersibility, adhesives, anti-static agents, and softeners. They are widely used in industries such as cosmetics, pharmaceuticals, chemical fibers, rubber, plastics, papermaking, painting, electroplating, pesticides, metal processing, and food processing. The properties vary depending on the relative molecular weight. From colorless and odorless viscous liquids to waxy solids, those with a molecular weight of 200-600 are liquids at room temperature, while those with a molecular weight above 600 gradually become semi-solid. The properties also differ with the average molecular weight. From colorless and odorless viscous liquids to waxy solids, as the molecular weight increases, their hygroscopicity decreases accordingly; Among them, PEG4000 and PEG6000 are commonly used to promote cell fusion or protoplast fusion and help organisms (such as yeast) uptake DNA during transformation.

PEG8000 Solution(50%, Sterilized) is mainly composed of PEG8000 and phosphate, and has undergone strict aseptic treatment. 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. PEG8000 Solution(50%, Sterilized) can be used as a fusion agent to obtain hybridoma cells for producing monoclonal antibodies. This reagent is only used in the field of scientific research and is not suitable for clinical diagnosis or other purposes.

Self Provided Materials

1. CO2 incubator, centrifuge
2. MEM medium, fetal bovine serum(FBS), HAT/HT/A Media Supplement, Trypsin digestion solution

Protocol(*for reference only*)

Note: If the solution turns into a gel like state, can heat it in a 37-60 ℃ water bath to restore solution state.

For single layer adherent cells:

1. Inoculate the hybrid precursor cells in the same quantity ($5x10^4$/ml), culture the cells in appropriate medium, and wait for the cells to adhere and expand to the density of the confluent sheet (80% confluence rate).
2. Absorb the culture medium, add 2ml of PEG8000 Solution(50%, Sterilized), gently rotate for 1 minute to cover all cells with PEG8000 solution, and let it stand for 1 minute.
3. Add 3ml of complete MEM medium to dilute PEG8000 solution, aspirate the diluted PEG8000 solution, and then add 5ml of MEM medium to wash the cells treated with PEG8000 once.
4. Remove the washing solution and add 5ml MEM medium. Incubate overnight at 37 ℃ with 5% CO2.
5. After 24-48 hours of cultivation, first remove the culture medium, then add trypsin digestion solution to treat the cells. After sufficient digestion, discard the trypsin digestion solution.
6. Cultivate and eliminate HPRT and TK deficient cells using HAT Media, discard the supernatant, resuspend the cells in complete culture medium supplemented with 1× HT and 1× A, fuse for 12-24 hours, and 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.

For suspension cells:

1. Mix 1ml (approximately $1×10^7$) of cells from two different parents, centrifuge at 800g for 10 minutes to precipitate the hybrid precursor cells, discard the supernatant, and let about 1ml remain. Gently tap the bottom of the tube or manually centrifuge the tube to mix the two types of cells and resuspend them.
2. Add 1ml PEG8000 Solution(50%, Sterilized) to the centrifuge tube and place it in a 37 ℃ water bath for 2 minutes.
3. Add 5ml of MEM culture medium containing 10% fetal bovine serum preheated at 37 ℃ in advance, dilute and stop the action of PEG8000, centrifuge 1000g for 5 minutes, discard the supernatant, add 5ml of

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complete MEM medium to dilute PEG8000 solution, aspirate the diluted PEG8000 solution, add 5ml of serum-free MEM medium, shake the centrifuge tube to resuspend the cells (do not damage the cells), centrifuge 1000g for 5 minutes, discard the supernatant, and repeat this step once.

4. Add HAT selective culture medium containing 20% fetal bovine serum, mix well, dilute the cell suspension with culture medium to 5×10^4/ml, inoculate in a 96 well plate (0.1ml per well) or other vessel, incubate overnight at 37 ℃ with 5% CO2, and select fusion cells after 24-48 hours.

Note

1. Pay attention to aseptic operation to avoid contamination by microorganisms.
2. Single layer adherent cells or suspended cells cultured in vitro can be fused, but the probability of success is higher for single layer adherent cells.
3. Please use the reagent as soon as possible after opening to avoid affecting the subsequent experimental results.
4. For your safety and health, please wear lab clothes and disposable gloves when operating.