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磷酸钙法细胞转染试剂盒

产品编号	产品名称	包装
C0508	磷酸钙法细胞转染试剂盒	>200次

产品简介：

- 碧云天生产的磷酸钙法细胞转染试剂盒(Calcium Phosphate Cell Transfection Kit)，在传统的磷酸钙细胞转染方法的基础上进行了改良，提高了转染效率，并降低了毒性。用磷酸钙法转染细胞，不仅可以瞬时表达，也可以筛选稳定株。
- HEK293或HEK293T细胞，即通常所谓的293细胞，是最适合磷酸钙法转染的细胞之一，优化条件后转染效率可以高达90%以上；通常很容易达到40-50%左右的转染效率。大多数常见的细胞例如Hela细胞、CHO细胞等也都适合磷酸钙法转染，但效率比293细胞要略低一些。
- 本试剂盒不仅适合于大多数贴壁细胞的转染，也适用于一些悬浮细胞的转染。
- 本试剂盒提供的试剂都经无菌处理，可以直接使用。
- 关于不同的细胞转染试剂的主要特点和差异方面的比较，以及如何选择适当的细胞转染试剂，可参考碧云天的相关网页：http://www.beyotime.com/support/transfection.htm。
- 本试剂盒足够转染不少于200个细胞样品。

包装清单：

产品编号	产品名称	包装
C0508-1	BBS溶液	20ml
C0508-2	氯化钙溶液	20ml
—	说明书	1份

保存条件：

-20°C保存，一年有效。

注意事项：

- 转染时，把DNA-氯化钙溶液加入到BBS溶液中，不要把BBS溶液加入到DNA-氯化钙溶液中。
- 高纯度的质粒是获得高转染效率的必要条件，要确保A260/A280大于1.8，并且电泳检测抽提到的质粒90%以上都是超螺旋。
- 在用磷酸钙法转染细胞时应使用浓度不高于5%的二氧化碳，二氧化碳的最佳浓度为3%左右。
- BBS溶液的pH值直接关系到转染效率，尽量避免把BBS溶液长时间暴露在空气中，以免被空气中的二氧化碳酸化。
- 转染时由于质粒不同、细胞不同，最佳的质粒用量需自行摸索。
- 本产品仅限于专业人员的科学研究用，不得用于临床诊断或治疗，不得用于食品或药品，不得存放于普通住宅内。
- 为了您的安全和健康，请穿实验服并戴一次性手套操作。

使用说明：

1. 对于贴壁细胞：

a. 将细胞培养于培养皿或培养板内，通常在铺板后1-2天内长到70-80%满为宜。后续说明都按6孔板内一个孔进行说明，如果有更多个孔或大小不相同的培养器皿，请自行换算。

b. 在转染前30-60分钟，吸去细胞培养液，加入新鲜的不含抗生素的完全培养液2毫升。**注意：**转染时最好使用新鲜配制且pH值经过精心调校的培养液；转染用的培养液可以在配制后分装冻存，使用时再解冻。

c. 取2-6微克待转染的质粒DNA (质粒总体积不宜超过20微升)，加入到100微升氯化钙溶液中，混匀。

d. 把DNA-氯化钙溶液加入到100微升BBS溶液中，混匀，室温孵育10-20分钟。此时不会产生可见的沉淀。

e. 把DNA-氯化钙-BBS混合物均匀滴加到整个6孔板内。在含5%二氧化碳的37°C细胞培养箱内培养。

f. 根据实验要求和磷酸钙对于不同细胞的毒性不同，在4-16小时后轻轻晃动培养板数次以充分悬浮一些磷酸钙沉淀，吸去含磷酸钙沉淀的培养液，加入2毫升新鲜的完全培养液，继续培养。

g. 通常在转染约24小时后就可以检测到转染基因的表达。

2. 对于悬浮细胞：

a. 离心收集悬浮细胞，用PBS洗涤一次。

b. 按照上面的步骤c)和d)制备DNA-氯化钙-BBS混合物。

c. 每10^6个细胞的沉淀，用100微升DNA-氯化钙-BBS混合物重新悬浮，室温放置20分钟。

d. 在一个6孔板孔内加入2毫升完全培养液，然后加入来自上一步的细胞-DNA-氯化钙-BBS混合物，混匀。
e. 在含5%二氧化碳的37°C细胞培养箱内培养。
f. 根据实验要求和磷酸钙对于不同细胞的毒性不同，在4-16小时后离心收集细胞，用PBS洗一次，然后用2毫升完全培养液重新悬浮细胞，继续培养。
g. 通常在转染约24小时后就可以检测到转染基因的表达。

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Version 2024.03.12