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细胞核蛋白与细胞浆蛋白抽提试剂盒

产品编号	产品名称	包装
P0028	细胞核蛋白与细胞浆蛋白抽提试剂盒	100次

产品简介：

- 在研究细胞时经常要研究细胞的不同组份，而研究得最多的两个细胞组份就是细胞核和细胞浆。分离细胞核蛋白和细胞浆蛋白，不仅可以用于研究蛋白在细胞内的定位，而且很多时候分离出来的核蛋白可以用于转录调控方面的研究，例如EMSA(也称gel shift)，footprinting等。
- 细胞核蛋白与细胞浆蛋白抽提试剂盒(Nuclear and Cytoplasmic Protein Extraction Kit)提供了一种比较简单、方便的从培养细胞或新鲜组织中抽提细胞核蛋白与细胞浆蛋白的方法。约90分钟就可以完成培养细胞的细胞核蛋白与细胞浆蛋白的分离。抽提得到的蛋白可以用于Western，EMSA，footprinting，报告基因检测以及酶活力测定等后续操作。
- 本试剂盒是通过细胞浆蛋白抽提试剂A和B，在低渗透压条件下，使细胞充分膨胀，然后破坏细胞膜，释放出细胞浆蛋白，然后通过离心得到细胞核沉淀。最后通过高盐的细胞核蛋白抽提试剂抽提得到细胞核蛋白。
- 对于细胞样品，如果每个样品的数量不超过二百万个细胞，本试剂盒可以抽提100个样品；对于组织样品，如果每个样品的重量不超过30毫克，本试剂盒可以抽提100个样品，如果每个样品约为30-60毫克，本试剂盒可以抽提50个样品。

包装清单：

产品编号	产品名称	包装
P0028-1	细胞浆蛋白抽提试剂A	20ml
P0028-2	细胞浆蛋白抽提试剂B	1ml
P0028-3	细胞核蛋白抽提试剂	5ml
—	说明书	1份

保存条件：

-20ºC保存，一年有效。

注意事项：

- 需自备PMSF。PMSF一定要在抽提试剂加入到样品中前2-3分钟内加入，以免PMSF在水溶液中很快失效。PMSF(ST506)可以向碧云天订购。
- 抽提蛋白的所有步骤都需在冰上或4ºC进行。
- 本试剂盒对于组织样品，仅适合于新鲜组织，对冻存过的组织抽提效果很差。可以抽提的组织样品数通常不足100个。
- 使用本试剂盒抽提到的细胞核蛋白与细胞浆蛋白均可直接用碧云天生产的BCA蛋白浓度测定试剂盒(P0009/P0010/P0010S/P0011/P0012/P0012S)测定蛋白浓度。但不适合用Bradford法测定蛋白浓度。
- 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. **准备溶液：**室温融解试剂盒中的三种试剂，溶解后立即放置在冰上，混匀。取适当量的细胞浆蛋白抽提试剂A备用，在使用前数分钟内加入PMSF，使PMSF的最终浓度为1mM。取适当量的细胞核蛋白抽提试剂备用，在使用前数分钟内加入PMSF，使PMSF的最终浓度为1mM。
2. **对于贴壁细胞：**用PBS洗一遍，用细胞刮子刮下细胞，或用EDTA溶液处理细胞使细胞不再贴壁很紧，并用移液器吹打下细胞。离心收集细胞，尽最大努力吸尽上清，留下细胞沉淀备用。尽量避免用胰酶消化细胞，以免胰酶降解需抽提的目的蛋白。
3. **对于悬浮细胞：**用PBS洗一遍，离心收集细胞，尽最大努力吸尽上清，留下细胞沉淀备用。
4. 每20微升细胞沉淀加入**200微升**添加了PMSF的细胞浆蛋白抽提试剂A。(对于二百万HeLa细胞，其细胞沉淀的体积大约为20微升或40毫克。)
5. 最高速剧烈Vortex 5秒，把细胞沉淀完全悬浮并分散开。(如果细胞沉淀没有完全悬浮并分散开，可以适当延长vortex时间。)
6. 冰浴10-15分钟。
7. 加入细胞浆蛋白抽提试剂B **10微升**。最高速剧烈Vortex 5秒，冰浴1分钟。
8. 最高速剧烈Vortex 5秒，4ºC 12,000-16,000g离心5分钟。
9. 立即吸取上清至一预冷的塑料管中，即为抽提得到的细胞浆蛋白。可以立即使用，也可以冻存。(千万不要触及沉淀，可以

在沉淀上方保留极小体积的上清，以免触及沉淀。每200万细胞用200微升本产品中的细胞浆蛋白抽提试剂A裂解后可获得的上清，其细胞浆蛋白浓度约为2-5mg/ml，不同细胞有所不同。)

10. 对于沉淀，完全吸尽残余的上清，加入**50微升**添加了PMSF的细胞核蛋白抽提试剂。(不吸尽上清会带来细胞浆蛋白的污染。)
11. 最高速剧烈Vortex 15-30秒，把细胞沉淀完全悬浮并分散开。然后放回冰浴中，每隔1-2分钟再高速剧烈Vortex 15-30秒，共30分钟。
12. 4ºC 12,000-16,000g离心10分钟。
13. 立即吸取上清至一预冷的塑料管中，即为抽提得到的细胞核蛋白。可以立即使用，也可以-70ºC冻存。每200万细胞用50微升本产品中的细胞核蛋白抽提试剂裂解后获得的上清，其细胞核蛋白浓度约为1.2-3.0mg/ml，不同细胞有所不同。
14. **对于新鲜组织：**
    a. 把组织尽可能切成非常细小的碎片。按照20:1的比例混合适当量的细胞浆蛋白抽提试剂A和B(例如200微升细胞浆蛋白抽提试剂A中加入10微升抽提试剂B)。并加入PMSF至最终浓度为1mM配制成组织匀浆液。按照每60mg组织加入200微升组织匀浆液的比例混合组织和组织匀浆液(对于不超过30mg的组织样品，仅需加入100微升组织匀浆液)，并在玻璃匀浆器内充分匀浆。匀浆需在冰浴或4ºC进行。
    b. 匀浆后把匀浆液转移到塑料离心管内，冰浴放置15分钟。
    c. 4ºC 1,500g离心5分钟。把上清转移至一预冷的塑料管中，为抽提得到的部分细胞浆蛋白。(吸上清时千万不要触及沉淀。)
    d. 对于沉淀，到了这一步已经充分匀浆，但很多细胞还没有破碎。接下去按照使用说明的步骤4开始操作，即把沉淀当做已经离心收集好的细胞沉淀操作，按照步骤4至步骤13抽提得到细胞浆蛋白和细胞核蛋白(**特别说明**：对于起始量为30-60mg的组织样品，后续直接按照步骤4-13操作即可；对于起始量不超过30mg的组织样品，后续步骤4-13中的溶液的用量须减半使用)。抽提得到的细胞浆蛋白可以和步骤14c中抽提得到的细胞浆蛋白合并。新鲜的肝脏组织用本产品裂解后获得的上清，其细胞浆蛋白浓度约为3-10mg/ml，细胞核蛋白浓度约为3-10mg/ml，不同状态的不同组织有所不同。

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