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细胞膜蛋白与细胞浆蛋白抽提试剂盒

产品编号	产品名称	包装
P0033	细胞膜蛋白与细胞浆蛋白抽提试剂盒	100次

产品简介：

- 碧云天的细胞膜蛋白与细胞浆蛋白抽提试剂盒(Membrane and Cytosol Protein Extraction Kit)提供了一种比较简单、方便地从培养细胞或组织中抽提细胞膜蛋白和细胞浆蛋白的方法。抽提的膜蛋白不仅包括质膜上的膜蛋白，也包括线粒体膜、内质网膜和高尔基体膜等上的膜蛋白。
- 本试剂盒通过匀浆适度破碎细胞，经低速离心去除细胞核和少数未破碎的细胞产生的沉淀，随后取上清高速离心获得细胞膜沉淀和含有细胞浆蛋白的上清，然后通过优化的膜蛋白抽提试剂从沉淀中抽提获取膜蛋白。
- 约90分钟即可完成培养细胞或组织的细胞膜蛋白与细胞浆蛋白的分离和抽提。抽提得到的蛋白可以用于SDS-PAGE，Western、酶活性测定等后续实验。
- 膜蛋白抽提试剂中含有蛋白酶抑制剂、磷酸酯酶抑制剂和EDTA等，后续不适合用于蛋白酶、磷酸酯酶等受这些抑制剂影响的酶的活性测定，但抽提获得的膜蛋白或细胞浆蛋白适合用于检测蛋白的磷酸化水平。
- 本试剂盒按照本说明书的操作步骤可以抽提100个细胞或组织样品。

包装清单：

产品编号	产品名称	包装
P0033-1	膜蛋白抽提试剂A	100ml
P0033-2	膜蛋白抽提试剂B	30ml
—	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。
- 使用本试剂盒抽提到的细胞膜蛋白与细胞浆蛋白均可直接用碧云天生产的BCA法蛋白浓度测定试剂盒(P0009/P0010/P0010S/P0011/P0012/P0012S)测定蛋白浓度。抽提获得的细胞膜蛋白不适合用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. **准备试剂：**室温融解并混匀膜蛋白抽提试剂 A 和 B，融解后立即置于冰浴上。取适量的膜蛋白抽提试剂 A 和 B 备用，在使用前数分钟内加入 PMSF，使 PMSF 的最终浓度为 1mM。
2. **准备细胞或组织样品：**
   a. **对于细胞**
      (a) **收集细胞**
      对于贴壁细胞：培养约2000-5000万细胞，用PBS洗一遍，用细胞刮子刮下细胞或用含有EDTA但不含胰酶的细胞消化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，吸除上清，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膜蛋白。
      对于悬浮细胞：培养约2000-5000万细胞，直接离心收集细胞，吸除上清，留下细胞沉淀备用。
      (b) **洗涤细胞：**用适量冰浴预冷的PBS轻轻重悬细胞沉淀，取少量细胞用于计数，剩余细胞4ºC，600g离心5分钟沉淀细胞。弃上清，随后4ºC，600g离心1分钟，以沉淀离心管管壁上的残留液体并进一步沉淀细胞，尽最大努力吸尽残留液体。
      (c) **细胞预处理：**把1毫升临用前添加了PMSF的膜蛋白抽提试剂A加入至2000-5000万细胞中，轻轻并充分悬浮细胞，冰浴放置10-15分钟。
   b. **对于组织：**取约100毫克组织，用剪刀尽量小心剪切成细小的组织碎片。加入1毫升临用前添加了PMSF的膜蛋白抽提试剂A，轻轻悬浮组织碎片，冰浴放置10-15分钟。注：如果组织样品比较少，也可以使用更少的组织量，例如30-50mg，后续试剂的用量及操作步骤不变；组织用量较少时，最后获得的膜蛋白也较少。
3. **细胞或组织样品的破碎及破碎效果的鉴定：**把细胞悬液或组织样品转移到一适当大小的冰浴预冷玻璃匀浆器中，匀浆约30-50

下。匀浆效果与细胞类型和组织类型相关，不同细胞或组织所需的匀浆次数有所不同，需自行优化。通常可以在匀浆30次后取约2-3微升细胞或组织匀浆液滴在盖玻片上并在显微镜下观察，如见细胞核周晕环(A shiny ring around the nuclei)或完整的细胞形态，说明细胞仍完整。如果有70-80%的细胞均无核周晕环和完整细胞形态，说明细胞已经充分破碎，则进行下一步实验。否则，重新匀浆10-30次直到细胞至少70%已经破碎。同时记录对于该细胞的匀浆次数，通常在后续实验时不必再摸索匀浆次数。另外需注意特定的匀浆次数和匀浆器也有关，需同时注意记录使用的是哪一个匀浆器。

注：如果没有适当的玻璃匀浆器，对于培养的细胞也可以采用冻融法来破碎细胞。把步骤2中的样品在液氮和室温依次反复冻融两次，然后取少量样品在显微镜下检测细胞破碎的程度。如果细胞破碎的程度不足70%，可增加冻融次数，直到细胞破碎的程度大于70%。

4. **去除细胞核和未破碎的细胞：**4ºC，700g离心10分钟，小心收集上清液至一新的离心管中。吸取上清时切勿接触沉淀！可以有约30-50微升上清液残留不予吸取，以保证吸取的上清液有较高的纯度。
5. **沉淀细胞膜碎片：**4ºC，14000g离心30分钟，以沉淀细胞膜碎片。
6. **收集细胞浆蛋白：**吸取上清即为细胞浆蛋白，可-70ºC保存备用。吸取上清时可以有30-50微升上清残留，以避免接触沉淀导致上清样品被污染。每5000万细胞使用本产品裂解可获得5-30mg细胞浆蛋白, 不同细胞有所不同。
7. **抽提膜蛋白：**4ºC，14000g离心10秒，尽最大努力吸尽上清。可以轻轻触碰到沉淀，甚至吸走很少量的沉淀。加入膜蛋白抽提试剂B 200微升(如有必要，也可以加大到300微升)，最高速剧烈Vortex 5秒重悬沉淀，冰浴5-10分钟。重复前述步骤的vortex和冰浴孵育1-2次，以充分抽提膜蛋白。随后，4ºC，14000g离心5分钟，收集上清即为细胞膜蛋白溶液。可-70ºC保存备用。对于一些有特殊用途的膜蛋白，可自行配制适当的膜蛋白抽提试剂进行膜蛋白抽提。每5000万细胞使用本产品裂解可获得0.3-3mg细胞膜蛋白, 不同细胞有所不同。

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