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细胞线粒体分离试剂盒

产品编号	产品名称	包装
C3601	细胞线粒体分离试剂盒	50-100次

产品简介：

- 细胞线粒体分离试剂盒(Cell Mitochondria Isolation Kit)是用于快速便捷地分离培养细胞线粒体的试剂盒。
- 本试剂盒在分离线粒体的同时可以获得去除线粒体的细胞浆蛋白，可用于研究细胞色素c等线粒体蛋白向胞浆的释放。
- 使用本试剂盒分离获得的线粒体纯度较高，并且绝大部分分离获得的线粒体都含有完整的内膜和外膜，并具有线粒体的生理功能。因此本试剂盒分离得到的线粒体可以用于线粒体的生理功能等方面的研究。例如可以使用碧云天的C2006线粒体膜电位检测试剂盒(JC-1)测定分离得到的线粒体的膜电位。
- 本试剂盒分离得到的线粒体也可以被试剂盒中的线粒体裂解液或其它适当裂解液裂解后用于SDS-PAGE、Western、双向电泳等蛋白分析。
- 本试剂盒提供了线粒体制备过程中匀浆程度的重要判断指标，即台盼蓝染色，使分离得到的线粒体的质量更加有保证。台盼蓝染色液为选用试剂，在实验条件成熟后可以不必使用。
- 本试剂盒提供了蛋白酶抑制剂PMSF，使匀浆时蛋白酶的活性被适当抑制，这样在获得线粒体的同时还可以获得有时有用的去除了大量线粒体的蛋白，在进行蛋白或酶活分析时可以作为对照。
- 如果每个样品的细胞数量为2000-5000万，本试剂盒可以处理50-100个样品。

包装清单：

产品编号	产品名称	包装
C3601-1	线粒体分离试剂	125ml
C3601-2	台盼蓝染色液	10ml
C3601-3	线粒体储存液	15ml
C3601-4	线粒体裂解液	15ml
C3601-5	PMSF(晶体)	For 1.5ml 100mM
C3601-6	PMSF(溶剂)	1.5ml
—	说明书	1份

保存条件：

-20ºC保存，一年有效。其中台盼蓝染色液也可以4ºC保存，PMSF(晶体)和PMSF(溶剂)在配制成100mM PMSF溶液前可以室温保存。

注意事项：

- 试剂盒中的试剂对于不同的实验目的不必全部使用。
- 如果不是用于制备线粒体蛋白样品，线粒体分离试剂和线粒体裂解液中不必加入PMSF。
- 如果用于制备线粒体蛋白样品，线粒体分离试剂和线粒体裂解液中需添加PMSF。PMSF一定要在线粒体分离试剂或线粒体裂解液加入到样品中前2-3分钟内加入，以免PMSF在水溶液中很快失效。
- 分离线粒体的所有步骤均需在冰上或4ºC进行，所用溶液需冰浴或4ºC预冷。
- 通常在分离线粒体时前后两次离心速度选取600g和11,000g，如果希望纯度更高，但对线粒体的得率要求不高，前后两次离心速度可以采用1000g和3500g。
- 使用本试剂盒中的线粒体储存液稀释或储存的线粒体样品应及时使用，以免线粒体膜电位受影响。如果不能及时使用，建议在-80ºC保存。冻存后的线粒体样品不推荐用于膜电位的检测，但可以用线粒体蛋白或核酸的相关检测。
- 如果出现本试剂盒中线粒体储存液不够用的情况，可以单独订购线粒体储存液(C3609)。
- 台盼蓝与PMSF对人体有毒，操作时请特别小心，并注意有效防护以避免直接接触人体或吸入体内。
- Bradford法蛋白浓度测定试剂盒(P0006)、Bradford蛋白浓度测定试剂盒(去垢剂兼容型)(P0006C)和BCA蛋白浓度测定试剂盒(P0009/P0010/P0010S/P0011/P0012/P0012S)可以向碧云天订购。
- 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各种溶液，融解后立即置于冰上并混匀。第一次使用时，把1.5ml PMSF(溶剂)加入到

PMSF(晶体)中，溶解并混匀，即得到1.5ml 100mM PMSF。配制好的100mM PMSF溶液-20ºC保存。如果最终目的是制备线粒体蛋白样品，根据样品数量，取适量线粒体分离试剂备用，在线粒体分离试剂加入到细胞样品中前数分钟内加入PMSF，使PMSF的最终浓度为1mM。同时，根据样品数量取适量线粒体裂解液备用，在线粒体裂解液加入到线粒体样品中前数分钟内加入PMSF，使PMSF的最终浓度为1mM。

2. **收集细胞：**
   对于贴壁细胞：用PBS洗一遍，用胰酶细胞消化液(Trypsin-EDTA Solution)消化细胞，100-200g，室温离心5-10分钟收集细胞。胰酶细胞消化液(C0201)可以向碧云天订购。
   对于悬浮细胞：直接离心收集细胞。
3. **洗涤细胞：**用冰浴预冷的PBS轻轻重悬细胞沉淀，取少量细胞用于计数，剩余细胞600g，4ºC离心5分钟沉淀细胞。弃上清。
4. **预处理：**加入1-2.5ml线粒体分离试剂或临用前添加了PMSF的线粒体分离试剂至2000-5000万细胞中，轻轻悬浮细胞，冰浴放置10-15分钟。
5. **匀浆：**把细胞悬液转移到一适当大小的玻璃匀浆器中，匀浆10-30下左右。
6. **匀浆效果的鉴定：**不同细胞不同匀浆器所需的匀浆次数有所不同，需自行优化。通常可以在匀浆10次后取约2微升细胞匀浆，加入30-50微升台盼蓝染色液，混匀后显微镜下观察台盼蓝染色阳性(蓝色)细胞的比例。如果阳性细胞比例不足50%，增加5次匀浆。随后再同前取样进行台盼蓝染色鉴定。当阳性比例超过50%时即可停止匀浆进入下一步。请勿过度匀浆，过度匀浆会导致线粒体的机械损伤。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。
7. 把细胞匀浆在600g，4ºC离心10分钟。
   注：如需获得纯度更高的线粒体，可以将此步骤的离心速度改为1000g，其它离心条件不变；获得更高纯度线粒体的缺点是相同数量细胞的线粒体抽提得率会下降。
8. 小心把上清转移到另一离心管中，在11,000g，4ºC离心10分钟。
   注：如需获得纯度更高的线粒体，可以将此步骤的离心速度改为3500g，其它离心条件不变；获得更高纯度线粒体的缺点是相同数量细胞的线粒体抽提得率会下降。
9. 小心去除上清。沉淀即为分离得到的细胞线粒体。
   注：如果希望获得去除线粒体的细胞浆蛋白，在本步骤需收集上清，并且在收集上清时注意勿触及沉淀。随后把收集的上清12,000g，4ºC离心10分钟。取上清即为去除线粒体的细胞浆蛋白。该细胞浆蛋白可以使用BCA法或Bradford法测定蛋白浓度。
10. **线粒体的使用：**
    a. 如果用于完整线粒体的功能或酶活性研究，初始2000-5000万细胞分离得到的线粒体样品中可以加入150-200微升线粒体储存液，重悬线粒体。
    b. 如果用于线粒体的蛋白分析，初始2000-5000万细胞分离得到的线粒体样品中可以加入150-200微升临用前添加了PMSF的线粒体裂解液裂解线粒体。裂解后的线粒体可以用于PAGE、Western、IP以及线粒体中的一些酶活性的测定等。裂解后的蛋白样品可以使用BCA法或去垢剂兼容型Bradford法(P0006C)测定蛋白浓度。
    c. 如果用于双向电泳，请使用适当的用于双向电泳的裂解液处理线粒体。

相关产品：

产品编号	产品名称	包装
C2006	线粒体膜电位检测试剂盒(JC-1)	>100次
C3601	细胞线粒体分离试剂盒	50-100次
C3606	组织线粒体分离试剂盒	50-100次
C3609	线粒体储存液	50ml

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