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组织线粒体分离试剂盒

产品编号	产品名称	包装
C3606	组织线粒体分离试剂盒	50-100次

产品简介：

- 组织线粒体分离试剂盒(Tissue Mitochondria Isolation Kit)是用于快速便捷分离动物组织线粒体的试剂盒。
- 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。
- 提供了两种不同的线粒体分离试剂，适用于不同的组织样品。
- 本试剂盒提供了用于组织消化的胰酶消化液，使对组织样品的处理更加便捷。
- 如果每个组织样品的重量为50-100mg，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
C3606-1	线粒体分离试剂A	60ml
C3606-2	线粒体分离试剂B	60ml
C3606-3	胰酶消化液	50ml
C3606-4	线粒体储存液	3ml
C3606-5	线粒体裂解液	15ml
—	说明书	1份

保存条件：

-20ºC保存，一年有效。其中胰酶消化液可以4ºC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。
- 如果用于制备蛋白样品，需自备PMSF，否则不必使用PMSF。PMSF(ST506)可以向碧云天订购。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。
- 使用说明中的操作步骤按照组织重量为50-100mg进行说明，如果组织块较大，可以按比例加大各溶液的使用量。
- 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)。
- 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，溶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，根据样品数量，取适量相应的线粒体分离试剂备用，在线粒体分离试剂加入到组织样品中前数分钟内加入PMSF，使PMSF的最终浓度为1mM。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. **从软组织(例如肝脏和脑)中分离线粒体：**
   a. 使用新鲜的动物组织，通常要求动物处死后不超过一小时，并且保存在冰上的组织。请勿使用经过冷冻保存的组织。
   b. 剪取一小块组织，重量约为50-100mg。在1.5ml离心管内对剪取的组织进行称重。用PBS洗涤组织一次。
   c. 把组织放在一个置于冰上的离心管或培养皿中，用剪刀或刀片把组织剪切成非常细小的组织碎片。
   d. 加入10体积预冷的线粒体分离试剂A或临用前添加了PMSF的线粒体分离试剂A，在冰浴上进行匀浆，匀浆10次左右。
      注：如果组织的重量为80mg，可以大致认为组织的体积接近80微升，此时需加入800微升线粒体分离试剂A。
   e. 把匀浆在600g，4ºC离心5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。
   f. 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。
   g. 小心去除上清。沉淀即为分离得到的线粒体。
      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。该细胞浆蛋白如果需要测定蛋白浓度，必须使用PBS或生理盐水至少稀释2倍，然后才能用BCA法或Bradford法进行检测(须特别注意，标准品也需要配制在含有相应比例线粒体分离试剂的PBS或生理盐水中)。
3. **从硬组织(例如心肌和骨骼肌)中分离线粒体：**
   a. 对于心肌组织采用线粒体分离试剂A，对于骨骼肌组织采用线粒体分离试剂B，对于其它类似组织可以优先考虑使用线粒体分离试剂A，如遇到效果不佳的情况可以试用线粒体分离试剂B。
   b. 使用新鲜的动物组织，通常要求动物处死后不超过一小时，并且保存在冰上的组织。请勿使用经过冷冻保存的组织。
   c. 剪取一小块组织，重量约为50-100mg。在1.5ml离心管内对剪取的组织进行称重。用PBS洗涤组织一次。
   d. 把组织放在一个置于冰上的离心管或培养皿中，用剪刀或刀片把组织剪切成非常细小的组织碎片。
   e. 加入10体积预冷的PBS，冰浴3分钟。注：如果组织的重量为80mg，可以大致认为组织的体积接近80微升，此时需加入800微升PBS。
   f. 600g离心10-20秒，沉淀组织样品，弃上清。
   g. 再加入8体积预冷的胰酶消化液，冰浴20分钟。
      注：如果组织的重量为80mg，此时需加入约640微升相应的胰酶消化液。
   h. 600g离心10-20秒，沉淀组织样品，弃上清。
   i. 加入2体积相应线粒体分离试剂，重悬组织，用于洗去残余的胰酶。
      注：如果组织的重量为80mg，此时需加入约160微升线粒体分离试剂。
   j. 600g离心10-20秒，沉淀组织样品，弃上清。
   k. 加入8体积预冷的相应线粒体分离试剂或临用前添加了PMSF的线粒体分离试剂，在冰浴上进行匀浆，匀浆20-30次。
      注：如果组织的重量为80mg，此时需加入约640微升相应的线粒体分离试剂。
   l. 把匀浆在600g，4ºC离心5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。
   m. 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。
   n. 小心去除上清。沉淀即为分离得到的线粒体。
      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。该细胞浆蛋白如果需要测定蛋白浓度，请参考步骤2g。
4. **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，可以加入适量线粒体储存液，重悬线粒体。通常每100mg组织获得的线粒体可以用40微升相应的线粒体储存液重悬，预期分离获得的线粒体样品的蛋白浓度为10-20mg/ml。该蛋白样品可以直接使用Bradford法测定蛋白浓度或离心沉淀后裂解再用BCA法测定蛋白浓度。注：线粒体储存液中含有的DTT会干扰BCA法测定蛋白浓度。
   b. 如果用于线粒体的蛋白分析，初始重量为50-100mg的组织样品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。

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