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细胞凋亡-DNA Ladder抽提试剂盒

产品编号	产品名称	包装
C0007	细胞凋亡-DNA Ladder抽提试剂盒	50次

产品简介：

- 碧云天生产的细胞凋亡－DNA Ladder抽提试剂盒，是针对细胞凋亡过程中产生的核小体间DNA链断裂而设计的。可以非常有效地抽提最小片段为180-200bp的DNA ladder，同时又可以抽提到50kb以上的基因组DNA。
- DNA ladder也称DNA fragmentation，是细胞凋亡的一个重要指标。通常观察到DNA ladder，就可以判定细胞发生了凋亡。
- 本试剂盒足够抽提50个细胞或组织样品。

包装清单：

产品编号	产品名称	包装
C0007-1	样品裂解液	30ml
C0007-2	蛋白酶K	130μl
C0007-3	10M 醋酸铵	6ml
C0007-4	TE	6ml
—	说明书	1份

保存条件：

-20ºC保存，一年有效。10M 醋酸铵和TE也可以室温保存。

注意事项：

- 需自备Tris平衡苯酚、氯仿和无水乙醇。
- 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. 样品收集

a. 对于组织样品：

切下组织，并剪切成小块，置液氮中冻结，研碎或捣碎。或直接冰浴上匀浆。

b. 对于贴壁细胞：

胰酶消化后，PBS或生理盐水洗一次，1000-2000g离心1-2分钟，弃上清，收集细胞。

c. 对于悬浮细胞：

1000-2000g离心1-2分钟，弃上清，收集细胞。

2. DNA ladder抽提

a. 每1毫升样品裂解液中加入5微升蛋白酶K，混匀。

b. 对于上述收集好的样品，每5毫克组织或者10^6个细胞中加入500微升添加了蛋白酶K的样品裂解液，Vortex混匀，充分裂解组织或细胞。

c. 50ºC水浴消化过夜(通常12-20小时皆可)。

d. 加入500微升Tris平衡苯酚(pH8.0)。

e. Vortex剧烈混匀，使有机相和水相充分混合，以达到抽提效果。4ºC，12,000g离心5分钟。

f. 缓慢吸出上层水相至另一洁净离心管中。注意勿触及中间层，中间层通常含有变性的蛋白等，并用等体积Tris平衡酚再抽提一次(同步骤e)。

g. 缓慢吸出上层水相至另一洁净离心管中。注意勿触及中间层，中间层通常含有变性的蛋白等，并用等体积氯仿再抽提一次(同步骤e)。

h. 慢慢吸出约300微升上清液，加入60微升10M醋酸铵和600微升无水乙醇，颠倒数次混匀，此时可见DNA沉淀产生。-20ºC冻存1小时，以充分沉淀小片段DNA。冻存过夜或-70ºC冻存效果更佳。

i. 4ºC，12, 000g 离心10分钟，弃上清。

j. 加入600微升70%乙醇，轻轻颠倒约2次。4ºC，12, 000g 离心10分钟，小心吸去上清。注意：70%乙醇洗涤的时候，千万注意避免损失一些细小的DNA沉淀，这些沉淀中大部分是你所需的DNA ladder。

k. 尽量吸除残余的乙醇，待看不到明显的液体时，立即加入50-100微升TE溶解DNA。注意：不可过分干燥基因组DNA沉淀，否则会极难溶解。如果发现DNA沉淀难以溶解，可以在4ºC用摇床缓慢摇动过夜，以溶解DNA沉淀。

l. 取部分抽提得到的DNA，1%琼脂糖凝胶电泳分析。如果细胞发生凋亡，就可以观察到典型的DNA ladder。电泳时一定要注意换用新鲜配制的电泳液，DNA凝胶也要用新鲜配制的电泳液配制并新鲜配制后使用。电泳时为获取最佳的电泳效果使ladder充分分开，电泳速度宜适当慢一些，凝胶宜适当长一些，而加样孔宜更加扁平一些。选取适当较薄的梳齿，往往会获得更好的ladder电泳效果。

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Version 2021.09.01