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Caspase 9活性检测试剂盒

产品编号	产品名称	包装
C1158	Caspase 9活性检测试剂盒	100次

产品简介：

- Caspase 9活性检测试剂盒(Caspase 9 Activity Assay Kit)是采用分光光度法检测细胞或组织裂解液中caspase 9酶活性或纯化的caspase 9酶活性的试剂盒。
- Caspase (Cysteine-requiring Aspartate Protease)是一个在细胞凋亡过程中起重要作用的蛋白酶家族。Caspase 9也称ICE-LAP6或Mch6，有时被写作caspase-9或caspase 9，是细胞凋亡信号转导过程中比较上游的一个caspase。线粒体释放细胞色素c以后，caspase 9可以和细胞色素c以及Apaf1形成复合物，同时被激活。激活的caspase 9可以激活细胞凋亡的最关键酶caspase 3，从而促进后续的细胞凋亡信号。Caspase 9的激活可以通过磷酸化进行调控。
- 本Caspase 9活性检测试剂盒是基于caspase 9可以催化底物Ac-LEHD-pNA (acetyl-Leu-Glu-His-Asp p-nitroanilide)产生黄色的pNA (p-nitroaniline)，从而可以通过测定吸光度来检测caspase 9的活性。pNA在405nm附近有强吸收。
- 试剂盒中提供了caspase 9催化产生的黄色产物pNA，可以作为定量caspase 9酶活性的标准品。
- 本试剂盒用酶标仪检测或容量不超过100μl的分光光度检测杯检测时，除标准曲线外可以检测100个样品。

包装清单：

产品编号	产品名称	包装
C1158-1	裂解液	30ml
C1158-2	检测缓冲液	10ml/瓶，共2瓶
C1158-3	Ac-LEHD-pNA (2mM)	200μl/管，共5管
C1158-4	pNA (10mM)	1ml
—	说明书	1份

保存条件：

-20°C保存，Ac-LEHD-pNA和pNA需避光保存。

注意事项：

- 须自备可以测定A405或A400的酶标仪或容量不超过100μl的分光光度检测杯及相应分光光度计。优先考虑测定A405，如有困难可以测定A400。
- Ac-LEHD-pNA需尽量避免反复冻融，请注意适当分装。
- 测定蛋白浓度需Bradford蛋白浓度测定试剂盒(P0006)，可向碧云天订购。建议样品用水稀释1倍后再用Bradford法测定蛋白浓度，以降低DTT对蛋白浓度测定的干扰。
- pNA（中文名为4-硝基苯胺）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。pNA（10mM）在4°C、冰浴等较低温度情况下会凝固而粘在离心管管底、管壁或管盖内，可以20-25°C水浴温育片刻至全部融解后使用。
- 本试剂盒的裂解液可以和碧云天生产的其它caspase活性检测试剂盒的裂解液通用，即本试剂盒裂解液制备的蛋白样品可以用于碧云天其它caspase活性检测试剂盒的检测。
- 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. 准备工作：

a. 裂解液溶解后混匀并置于冰浴上备用。
b. 检测缓冲液溶解后混匀并置于冰浴上备用。

2. 测定pNA标准曲线：

a. 标准品稀释液的配制：按照每0.9ml检测缓冲液加入0.1ml裂解液的比例配制适量的标准品稀释液。
b. 试剂盒提供的pNA (10mM)用标准品稀释液稀释为0、10、20、50、100和200μM，作为标准品。
c. 每个浓度取100μl用酶标仪进行检测，或取适当量用容量不超过100μl的分光光度检测杯进行检测，测定A405。
d. 每一个标准品的A405减去不含pNA的空白对照的A405计算出实际的因pNA而导致的吸光度，并制作出pNA浓度相对于A405的标准曲线。pNA标准曲线可以参考图1，在0-200μM范围内存在良好的线性关系。

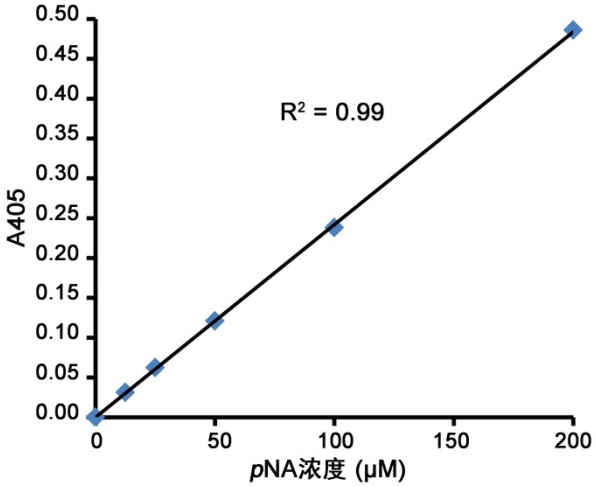

图1. pNA标准曲线。实测数据可能因实验条件、检测仪器等的不同而存在差异，图中数据仅供参考。

3. 样品的收集：

a. **对于悬浮细胞：**把没有诱导凋亡的对照样品和诱导凋亡的样品，600g 4°C离心5分钟收集细胞，小心吸除上清，同时确保尽量没有细胞被吸除，PBS洗涤一次。同前吸尽上清后，按照每200万细胞加入100微升裂解液的比例加入裂解液（如果裂解不充分，可以把裂解液的用量提高至150或200微升），重悬沉淀，冰浴裂解15分钟。下转步骤3d。

b. **对于贴壁细胞：**吸取细胞培养液，备用。用胰酶消化贴壁细胞，并收集至备用的细胞培养液中。600g 4°C离心5分钟收集细胞，小心吸除上清，同时确保尽量没有细胞被吸除，PBS洗涤一次。同前吸尽上清后，按照每200万细胞加入100微升裂解液的比例加入裂解液（如果裂解不充分，可以把裂解液的用量提高至150或200微升），重悬沉淀，冰浴裂解15分钟。下转步骤3d。

c. **对于组织样品：**按照每3-10mg组织加入100微升裂解液的比例加入裂解液，在冰浴上用玻璃匀浆器匀浆。然后把匀浆液转移到1.5ml离心管中，冰浴再裂解5分钟。

d. 4°C 16,000-20,000g离心10-15分钟。

e. 把上清转移到冰浴预冷的离心管中。

f. 立即测定caspase 9的酶活性或-70°C保存样品。同时可以取少量样品用Bradford法测定蛋白浓度，尽量使蛋白浓度达到1-3mg/ml，相当于每10微升待测样品中至少含有10-30μg蛋白。如果细胞较小，可以适当增加细胞的用量。

4. Caspase 9酶活性的检测：

a. 取出适量的Ac-LEHD-pNA (2mM)，置于冰浴上备用。

b. 如下设置反应体系：

	空白对照	样品
检测缓冲液	40μl	40μl
待测样品	0μl	50μl
裂解液	50μl	0μl
Ac-LEHD-pNA (2mM)	10μl	10μl
总体积	100μl	100μl

注意：在设置反应体系时先加检测缓冲液，再加待测样品，适当混匀，注意避免在混匀时产生气泡。随后再加入 10μl Ac-LEHD-pNA (2mM)。

c. 加入 Ac-LEHD-pNA (2mM)后混匀，注意避免在混匀时产生气泡。37°C 孵育 60-120 分钟。发现颜色变化比较明显时即可测定 A405。如果颜色变化不明显，可以适当延长孵育时间，甚至可以孵育过夜。

d. 样品的 A405 扣除空白对照的 A405，即为样品中 caspase 9 催化产生的 pNA 产生的吸光度。通过同步骤 1 中获得的标准曲线的对比就可以计算出样品中催化产生了多少量的 pNA。

e. 参考 Chemicon 公司的 caspase 9 酶活力单位的定义：One unit is the amount of enzyme that will cleave 1.0nmol of the colorimetric substrate Ac-LEHD-pNA per hour at 37°C under saturated substrate concentrations。即一个酶活力单位定义为当底物饱和时，在 37°C 一个小时内可以剪切 1nmol Ac-LEHD-pNA 产生 1nmol pNA 的 caspase 9 的酶量。这样就可以计算出样品中含有多少个酶活力单位的 caspase 9。说明：在本试剂盒的检测体系中，底物的起始浓度为 0.2mM，此时底物是饱和的，对于许多样品而言在 37°C 孵育 2 个小时以内底物都是饱和的；对于样品中 caspase 9 酶活力特别高的情况，须用裂解液适当稀释样品后再进行测定。

f. 用Bradford法检测待测样品中的蛋白浓度(由于裂解液中含有较高浓度的DTT，不适合采用BCA法进行蛋白浓度测定)。这样就可以计算出一个样品单位重量蛋白中所含的caspase 9的酶活力单位。

常见问题：

1. 测定出的A405过低：

a. 样品中蛋白含量太低，裂解样品时需设法使样品中的蛋白浓度至少达到1-3mg/ml。

b. 样品中激活的caspase水平很低。首先确认凋亡现象是否明显，如果凋亡比较明显并且确认该caspase是可以被激活的，可以适当调节诱导细胞凋亡的时间，希望能找到一个caspase激活比较强的时间点，这样就可以检测出该caspase的激活。可以作一时间曲线，例如诱导凋亡0、2、4、8、16和24小时，或0、1、2、4、8和16小时，或0、1、2、4、6和8小时等。具体的诱导凋亡时间需根据具体情况而定。

2. 测定出的A405过高或者样品量不足：

测定出来的A405读数过高时，可以参考下表的反应体系适当减少样品的用量；样品量不足时也可以参考下表减少样品的用量。

	空白对照	样品
检测缓冲液	40μl	40μl
待测样品	0μl	xμl
裂解液	50μl	(50-x) μl
Ac-LEHD-pNA (2mM)	10μl	10μl
总体积	100μl	100μl

说明：其中x不超过50，其余检测方法同上面的使用说明所述。

相关产品：

产品编号	产品名称	包装
C1101	Caspase 1 活性检测试剂盒	20次
C1102	Caspase 1 活性检测试剂盒	100次
C1107	Caspase 2 活性检测试剂盒	20次
C1108	Caspase 2 活性检测试剂盒	100次
C1115	Caspase 3 活性检测试剂盒	20次
C1116	Caspase 3 活性检测试剂盒	100次
C1121	Caspase 4 活性检测试剂盒	20次
C1122	Caspase 4 活性检测试剂盒	100次
C1135	Caspase 6 活性检测试剂盒	20次
C1136	Caspase 6 活性检测试剂盒	100次
C1151	Caspase 8 活性检测试剂盒	20次
C1152	Caspase 8 活性检测试剂盒	100次
C1157	Caspase 9 活性检测试剂盒	20次
C1158	Caspase 9 活性检测试剂盒	100次
P0006	Bradford蛋白浓度测定试剂盒	1000次

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