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## Caspase 8活性检测试剂盒

产品编号	产品名称	包装
C1151	Caspase 8活性检测试剂盒	20次

### 产品简介:

- Caspase 8活性检测试剂盒(Caspase 8 Activity Assay Kit)是采用分光光度法检测细胞或组织裂解液中caspase 8酶活性或纯化的caspase 8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 8也称FLICE、MACH或Mch5, 有时被写作caspase-8或caspase 8, 通常以酶原的形式存在, 在细胞凋亡的信号转导过程中被激活。Caspase 8被认为是细胞凋亡信号转导过程中比较上游的一个caspase。在Fas-receptor和TNFR-1介导的细胞凋亡过程中caspase 8被激活, 形成一个由p18和p10组成的二聚体, 进一步激活下游的caspase 4, caspase 6, caspase 9和caspase 10。
- 本Caspase 8活性检测试剂盒是基于caspase 8可以催化底物Ac-IETD-pNA (acetyl-Ile-Glu-Thr-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 8的活性。pNA在405nm附近有强吸收。
- 试剂盒中提供了caspase 8催化产生的黄色产物pNA, 可以作为定量caspase 8酶活性的标准品。
- 本试剂盒用酶标仪检测或容量不超过100 $\mu$ l的分光光度检测杯检测时, 除标准曲线外可以检测20个样品。

### 包装清单:

产品编号	产品名称	包装
C1151-1	裂解液	8ml
C1151-2	检测缓冲液	8ml
C1151-3	Ac-IETD-pNA (2mM)	200 $\mu$ l
C1151-4	pNA (10mM)	200 $\mu$ l
—	说明书	1份

### 保存条件:

-20 $^{\circ}$ C保存, Ac-IETD-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100 $\mu$ l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-IETD-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 $^{\circ}$ 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 $^{\circ}$ 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:

- 裂解液溶解后混匀并置于冰浴上备用。
- 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:

- 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。
- 把试剂盒提供的pNA (10mM)用标准品稀释液稀释为0、10、20、50、100和200 $\mu$ M, 作为标准品。
- 每个浓度取100 $\mu$ l用酶标仪进行检测, 或取适当量用容量不超过100 $\mu$ l的分光光度检测杯进行检测, 测定A405。
- 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0-200 $\mu$ M范围内存在良好的线性关系。

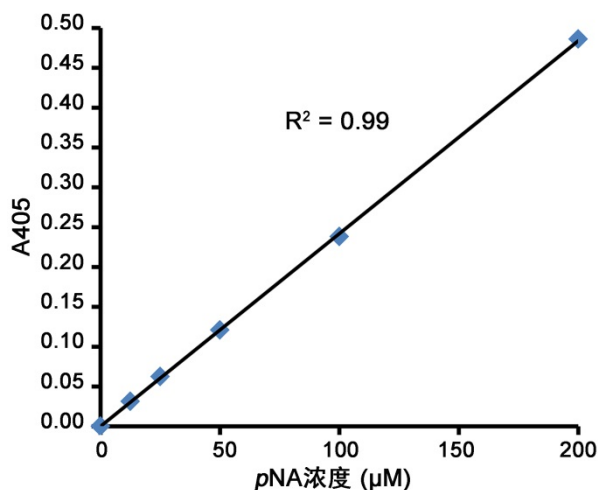


图1. pNA标准曲线。实测数据可能因实验条件、检测仪器等的不同而存在差异，图中数据仅供参考。

### 3. 样品的收集:

- 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。
- 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。
- 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。
- 4°C 16,000-20,000g离心10-15分钟。
- 把上清转移到冰浴预冷的离心管中。
- 立即测定caspase 8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 8酶活性的检测:

- 取出适量的Ac-IETD-pNA (2mM)，置于冰浴上备用。
- 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-IETD-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-IETD-pNA (2mM)。

- 加入Ac-IETD-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。
- 样品的A405扣除空白对照的A405，即为样品中caspase 8催化产生的pNA产生的吸光度。通过同步步骤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。
- 参考Chemicon公司的caspase 8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-IETD-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-IETD-pNA产生1nmol pNA的caspase 8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 8。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 8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。
- 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 8的酶活力单位。

### 常见问题:

#### 1. 测定出的A405过低:

- 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。
- 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-IETD-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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