



碧云天生物技术/Beyotime Biotechnology  
订货热线: 400-1683301 或 800-8283301  
订货 e-mail: order@beyotime.com  
技术咨询: info@beyotime.com  
网址: http://www.beyotime.com

## BCA蛋白浓度测定试剂盒

产品编号	产品名称	包装
P0012S	BCA蛋白浓度测定试剂盒	200次

### 产品简介:

- BCA蛋白浓度测定试剂盒(BCA Protein Assay Kit)是根据目前世界上最常用的两种蛋白浓度检测方法之一BCA法研制而成, 实现了蛋白浓度测定的简单、高稳定性、高灵敏度和高兼容性。
- 灵敏度高, 检测浓度下限达到25 $\mu$ g/ml, 最小检测蛋白量达到0.5 $\mu$ g, 待测样品体积为1-20 $\mu$ l。
- 在50-2000 $\mu$ g/ml浓度范围内有较好的线性关系。
- BCA法测定蛋白浓度不受绝大部分样品中的化学物质的影响, 可以兼容样品中高达5%的SDS, 5%的Triton X-100, 5%的Tween20、60、80。但本试剂盒受螯合剂和略高浓度的还原剂的影响, 需确保EDTA低于10mM, 无EGTA, 二硫苏糖醇(DTT)低于1mM,  $\beta$ -巯基乙醇( $\beta$ -Mercaptoethanol)低于0.01%。不适用BCA法时建议试用碧云天生产的Bradford蛋白浓度测定试剂盒(P0006)。
- BCA蛋白浓度测定试剂盒对样品中各种物质的详细的兼容性, 请参见碧云天如下网页:  
<http://www.beyotime.com/Compatibility Chart For BCA Kit.pdf>
- 每个试剂盒可以检测200个样品。

### 包装清单:

产品编号	产品名称	包装
P0012S-1	BCA试剂 A	40ml
P0012S-2	BCA试剂 B	1.2ml
P0012S-3	蛋白标准(BSA)	20mg
P0012S-4	蛋白标准配制液	1ml
—	说明书	1份

### 保存条件:

室温保存。蛋白标准配制成溶液后-20 $^{\circ}$ C冻存。

### 注意事项:

- 需酶标仪一台, 测定波长为540-595nm之间, 562nm最佳。需96孔板。如果没有酶标仪, 也可以使用普通的分光光度计测定, 但测定时, 需根据比色皿的最小检测体积, 适当加大BCA工作液的用量使不小于最小检测体积, 样品和标准品的用量可相应按比例放大也可不变。使用分光光度计测定蛋白浓度时, 每个试剂盒可以测定的样品数量可能会显著减少。
- 如发现样品稀释液或裂解液本身就有较高背景, 请试用碧云天生产的Bradford蛋白浓度测定试剂盒(P0006)。
- 为了加快BCA法测定蛋白浓度的速度可以适当用微波炉加热, 但是切勿过热。
- EDTA浓度必须小于10mM, 不兼容EGTA。不适用BCA法时, 请试用碧云天生产的Bradford蛋白浓度测定试剂盒(P0006)。
- 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. 蛋白标准品的准备

- 取0.8ml蛋白标准配制液加入到一管蛋白标准(20mg BSA)中, 充分溶解后配制成为**25mg/ml**的蛋白标准溶液。配制后可立即使用, 也可以-20 $^{\circ}$ C长期保存。
- 取适量25mg/ml蛋白标准, 稀释至终浓度为**0.5mg/ml**。例如取20 $\mu$ l 25mg/ml蛋白标准, 加入980 $\mu$ l稀释液即可配制成为0.5mg/ml蛋白标准。蛋白样品在什么溶液中, 标准品也宜用什么溶液稀释。但是为了简便起见, 也可以用0.9% NaCl或PBS稀释标准品。稀释后的0.5mg/ml蛋白标准可以-20 $^{\circ}$ C长期保存。

#### 2. BCA工作液配制

根据样品数量, 按50体积BCA试剂A加1体积BCA试剂B(**50:1**)配制适量BCA工作液, 充分混匀。例如5ml BCA试剂A加100 $\mu$ l BCA试剂B, 混匀, 配制成为5.1ml BCA工作液。BCA工作液室温24小时内稳定。

#### 3. 蛋白浓度测定

- 将**标准品**按0、1、2、4、8、12、16、20 $\mu$ l加到96孔板的标准品孔中, 加标准品稀释液**补足到20 $\mu$ l**, 相当于标准品浓度分别为

- 0、0.025、0.05、0.1、0.2、0.3、0.4、0.5mg/ml。
- b. 加适当体积样品到96孔板的样品孔中。如果样品不足20 $\mu$ l，需加标准品稀释液补足到20 $\mu$ l。请注意记录样品体积。
- c. 各孔加入200 $\mu$ l BCA工作液，37 $^{\circ}$ C放置20-30分钟。  
注：也可以室温放置2小时，或60 $^{\circ}$ C放置30分钟。BCA法测定蛋白浓度时，颜色会随着时间的延长不断加深。并且显色反应会因为温度升高而加快。如果浓度较低，适合在较高温度孵育，或适当延长孵育时间。
- d. 用酶标仪测定A562，或540-595nm之间的其他波长的吸光度。
- e. 根据标准曲线和使用的样品体积计算出样品的蛋白浓度。

#### 常见问题：

##### 1. 测定标准曲线时发现随着标准品浓度的增加吸光度或颜色没有明显变化。

可能的原因是样品中含有严重干扰BCA法测定蛋白浓度的物质，详细的BCA法的兼容性列表请参考碧云天如下网页：  
<http://www.beyotime.com/Compatibility Chart For BCA Kit.pdf>。

##### 2. 是否每次测定时都需要做标准曲线？

建议每次测定时都做标准曲线。因为BCA法测定时颜色会随着时间的延长不断加深，并且显色反应的速度和温度有关，所以除非精确控制显色反应的时间和温度，否则如需精确测定宜每次都做标准曲线。

#### 相关产品：

产品编号	产品名称	包装
P0006	Bradford蛋白浓度测定试剂盒	1000次
P0006C	Bradford蛋白浓度测定试剂盒(去垢剂兼容型)	800次
P0007	蛋白标准(5mg/ml BSA)	1ml
P0009	BCA蛋白浓度测定试剂盒(增强型)	5000次
P0010	BCA蛋白浓度测定试剂盒(增强型)	500次
P0010S	BCA蛋白浓度测定试剂盒(增强型)	200次
P0011	BCA蛋白浓度测定试剂盒	5000次
P0012	BCA蛋白浓度测定试剂盒	500次
P0012S	BCA蛋白浓度测定试剂盒	200次

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