蛋白质定量试剂盒 (BCA 法) P1511

描述：Bicinchoninic acid (BCA)法是近来广为应用的蛋白定量方法。其原理与Lowery法蛋白定量相似，即在碱性环境下蛋白质与Cu^{2+}络合并将Cu^{2+}还原成Cu^{1+}。BCA与Cu^{1+}结合形成稳定的紫蓝色复合物，在562 nm处有高的光吸收值并与蛋白质浓度成正比，据此可测定蛋白质浓度。与Lowery法相比，BCA蛋白测定方法灵敏度高，操作简单，试剂及其形成的紫蓝色复合物稳定性俱佳，并且受干扰物质影响小。与Bradford法相比，BCA法的显著优点是不受去垢剂的影响。

组成与储存：(1) BCA Reagent 100 ml，室温保存；(2) Cu Reagent 2 .5ml，室温保存；(3) BSA standard 4 mg/ml 1 ml，−20℃ 冻存。12 个月有效。可进行 500 次微板(microplate)测定或 100 次 1 ml 比色杯测定。

所需设备：比色计、酶标仪或微板比色仪，最佳工作波长 562 nm，可在 540-590 nm 之间。

工作溶液(Working Reagent, WR)配制：将 50 体积 BCA Reagent 与 1 体积 Cu Reagent 混合即为 WR 工作试剂，呈嫩绿色；室温 1 周内稳定。

标准蛋白溶液配制：用双蒸水、0.9%生理盐水、PBS 或与待测蛋白样品匹配的缓冲液进行倍比稀释: 20 µl 4000 µg/ml BSA + 30 µl 稀释溶液(H_2O/PBS/0.9%NaCl) = 50 µl (BSA=1600 µg/ml)，从中取 25 µl 连续倍比稀释，得到 BSA 标准溶液 1600、800、400、200、100、50、25 µg/ml，各 25 µl。通常，样品蛋白浓度不会太高，也可以预先稀释待测样品，可以省略 1600 µg/ml 标准管而直接从 800 或 1000 µg/ml 开始，能节省标准蛋白用量。

蛋白浓度测定：

蛋白质浓度线性检测范围为 10-2000 µg/ml。标准测定时，用 1 cm 光程玻璃或塑料比色皿，反应终体积 1.1 ml，比色计测定。微板测定时，用 96 孔板，反应终体积 225 µl，用酶标仪、微板比色仪测定。

1. 标准测定：将 0.05~0.1 ml 标准品或待测样本与 1 ml WR 工作溶液混合。
   微板测定：将 25 µl 标准品或待测样本与 200 µl WR 工作溶液混合。
2. 37ºC 反应 30min；也可 25ºC 室温 2 小时或过夜。60ºC 30 min 反应可增加检测灵敏度至 5-250µg/ml。
3. 将反应管冷却至室温。测定562 nm (可在540-590 nm之间)光密度(OD)值。
4. 绘制标准曲线。X轴为BSA标准蛋白浓度(mg/ml或µg/ml)，Y轴为各标准管对应的OD562值。用Excel拟合曲线并计算蛋白浓度。

表 1 标准测定和微板测定方案的加样量和比例

		微板(microplate)测定方案		标准比色杯测定方案	
标号	蛋白浓度 (µg/ml)	标准或待测蛋白体积 (µl)	WR 工作试剂(µl)	标准或待测蛋白体积 (ml)	WR 工作试剂(ml)
1	0	25	200	0.05-0.1	1
2	25	25	200	0.05-0.1	1
3	50	25	200	0.05-0.1	1
4	100	25	200	0.05-0.1	1
5	200	25	200	0.05-0.1	1
6	400	25	200	0.05-0.1	1
7	800	25	200	0.05-0.1	1
8	1600	25	200	0.05-0.1	1
待测样品		25	200	0.05-0.1	1

注意事项

1. 37ºC 30min 或 25ºC 室温反应 2 小时对测量较为便利，但严格来讲此时反应尚未达到终点，通常每 10 min OD562 值升高约 2.3%。然而，通常在 10min 内可以测定 30 管并不会明显影响测定精度。
2. BCA 法检测范围为 20-2000 µg/ml。检测 0.5~10 µg/ml 高度稀释蛋白样品应采用 Bradford 法蛋白质

定量试剂盒(# P1510)。60ºC 30 min 反应可增加检测灵敏度至 5~250μg/ml。

3. BCA法检测样品中含有脂类物质时光吸收值会偏高。样品中EDTA或葡萄糖浓度大于10 mM不能使用BCA方法，葡萄糖浓度大于10 mM时可用改良Lowry法蛋白定量试剂盒 #P1512，EDTA大于10 mM的样品可用Bradford法蛋白质定量试剂盒(# P1510)。另外，蛋白质样品经液体样品蛋白抽提试剂(#P1255)沉淀后，可彻底去除干扰BCA法、Bradford法、和Lowry法蛋白测定的物质。
4. 欲使测量能耐受下面表 2 所提示的最大干扰物质浓度，并保持测量精度，应在蛋白标准管中加入相应浓度的干扰物质，但会给操作带来不便。
5. 可测量吸附于固相支持物乳酶标板、琼脂糖、亲和层系凝胶上的蛋白。
6. 每次测定应该重新测定并制作标准曲线。

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Smith P et al, 1995, Measurment of protein using bicinchiconic acid, Anal. Biochem. 150, 76-85

表2 BCA法物质干扰及耐受的最大浓度

Buffer Systems	Sodium phosphate 25 mM
Bicine, pH 8.4 20 mM	Sucrose 40%
Bis-Tris, pH 6.5 33 mM	Sodium ortho-Vanadate in PBS, pH 7.2, 1 mM
Calcium chloride in TBS, pH 7.2 10 mM	Urea 3 M
CHES, pH 9.0 100 mM	**Chelating agents**
Cobalt chloride in TBS, pH 7.2 0.8 M	EDTA 10 mM
Ferric chloride in TBS, pH 7.2 10 mM	EGTA,any level, not compatible
HEPES 100 mM	Sodium citrate 200 mM
MOPS, pH 7.2 100 mM	**Detergents**
Nickel chloride in TBS 10 mM	Brij-35 5%
PBS; no interference	Brij-52 1%
NaCl (0.15 M), pH 7.2, no interference	CHAPS 5%
PIPES, pH 6.8 100 mM	CHAPSO 5%
Sodium acetate, pH 4.8 200 mM	Deoxycholic acid 5%
Sodium citrate, pH 4.8 or pH 6.4 200 mM	Nonidet P-40 (Igepal CA-630) 5%
Tricine, pH 8.0 25 mM	SDS 5%
Triethanolamine, pH 7.8 25 mM	Span 20 1%
Tris 250 mM	Triton X-100 5%
TBS buffer, no interference	Triton X-114 1%
1 x SDS-PAGE loading buffer, no interference	Tween-20 5%
Zinc chloride (10 mM) in TBS, pH 7.2, 10 mM	Tween-60 5%
Buffer Additives	Tween-80 5%
Ammonium sulfate 1.5 mM	Zwittergents 1%
Aprotinin 10 mg/L	**Reducing & Thiol Containing Agents**
Glucose 10 mM	Dithioerythritol (DTE) 1 mM
Glycerol 10%	Dithiothreitol (DTT) 1 mM
Guanidine•HCl 4 M	2-Mercaptoethanol 1 mM
HCl 100 mM	Tributyl Phosphine 0.01%
Imidazole 50 mM	**Solvents**
Leupeptin 10 mg/L	Acetone 10%
PMSF 1 mM	Acetonitrile 10%
Sodium azide 0.20%	DMF 10%
Sodium bicarbonate 100 mM	DMSO 10%
Sodium chloride 1 M	Ethanol 10%
Sodium hydroxide 100 mM	Methanol 10%

目前，使用普利莱 BCA 蛋白定量试剂盒发表的 SCI 文章已超过百篇，部分文章如下，供参考。

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