BCA Protein Assay (Pierce)

BCA assay: geeignet für geringe Proteinmengen!!!

Die Proteine bilden mit Cu2+-Ionen in alkalischer Lösung einen Komplex (Biuret-Reaktion). Die Cu2+-Ionen des Komplexes werden vermutlich zu Cu2+-Ionen reduziert, die mit Bicinchoninsäure (BCA) einen violetten Komplex bilden.
Protein concentrations between 5-250 µg/ml can be determined with this protocol.
(By modifying the protocol you can change the range to 20-2000 µg/ml. See data sheet.)

BSA dilutions for standard curve

tube	BSA conc. (µg/ml)	BSA-stock (2mg/ml), µl	A. bidest., µl	source of BSA µl
1	250	100	700	-
2	125	-	400	400 from tube 1
3	50	-	450	300 from tube 2
4	25	-	400	400 from tube 3
5	5	-	400	100 from tube 4
6 (“blank”)	0	-	400	-

50 µl from each dilution will be used for the assay procedure.

Working Reagent

Mix solution A with solution B as follows: 50 (**A**) :1 (**B**). Mix well to receive a light green reagent. You need 1 ml per sample. Mix only as much as you need for your samples (standards + “unknowns”, use duplicates for each sample).

In case the protein is dissolved in Laemmli sample buffer you **must** get rid of the EGTA which is included in the buffer as it strongly interferes with the assay. The easiest way is to precipitate the protein with acetone.

Acetone precipitation

50 µl sample is precipitated by adding 200 µl of ice-cold acetone (-20°C), vortex and leave at –20°C for 30 min. Centrifuge for 10 min at maximum speed and remove the supernatant. Leave test tube open for 30 min. Resolve the pellet in 50 µl of A. bidest. by vortexing. Repeat the precipitation step once. Include one sample wich contains the interfering buffer without protein (control). The absorbance of this sample will be subtracted from the absorbances obtained from the “unknowns”.

Assay procedure

Add 1 ml Working Reagent to 50µl of samples (standard and unknown samples), vortex, incubate for 30 min at 60°C. Cool down to room temperature and measure OD of all samples at 562 nm within 10 minutes. Zero the spectrophotometer with water.
Subtract the “blank” (standard tube #6) from the absorbances of the standard dilutions (#1-#5). Subtract the absorbance of the control from the “unknowns”.

The protocol may be modified in many ways including incubation time and temperature. See data sheet for instructions!

Protein Assay, Bradford

Assay not appropriate for low amounts of protein!!!

The assay to determine the total protein concentration is based on the protein binding property of the dye Coomasssie Brilliant Blue G250, which results in a shift of λ max from 465nm to 595nm. Measuring the OD at 595nm of the protein solution determines the final protein concentration.
The effective range of this assay is between 0.1-5.0 mg/ml total protein, for lower protein concentration the BCA-kit is recommended.

To determine the total Protein concentration of a sample a BSA standard curve has to be set up with either: 0 mg/ml; 0,25 mg/ml; 0,5 mg/ml; 0,75 mg/ml; 1mg/ml; 1,25 mg/ml, for low range or 0,1mg/ml; 0,5 mg/ml; 1 mg/ml; 2 mg/ml; 5mg/ml for high range protein concentrations.

Method:

- always work in duplicats
- mix 1ml of Bradford solution with 10µl protein and incubate for 5min to 1h
- first measure the standard curve data, followed by the protein lysates of interest, properly diluted (e.g., 1:1, 1:2 and 1:10), calculate the data via the graph

Bradford solution:

Dissolve 100mg Coomassie Blue G250 in 50ml 95% Ethanol and 100ml 85% H_3PO_4. Fill up to 1000ml with A.bidest and filter.
The solution is stored in a brown bottle at 4°C.

Maximum useful concentrations of Substances known to interfere with the Bradford assay:

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Table 2. Compatible Substance Concentrations in the Coomassie Assay (see text for details).

Substance	Compatible Concentration
Salts/Buffers	
ACES, pH 7.8	100 mM
Ammonium sulfate	1.0 M
Asparagine	10 mM
Bicine, pH 8.4	100 mM
Bis-Tris, pH 6.5	100 mM
Borate (50 mM), pH 8.5 (# 28384)	undiluted
B-PER® Reagent (#78248)	1/2 dilution*
Calcium chloride in TBS, pH 7.2	10 mM
Na-Carbonate/Na-Bicarbonate (0.2 M), pH 9.4 (#28382)	undiluted
Cesium bicarbonate	100 mM
CHES, pH 9.0	100 mM
Na-Citrate (0.6 M), Na-Carbonate (0.1 M), pH 9.0 (#28388)	undiluted
Na-Citrate (0.6 M), MOPS (0.1 M), pH 7.5 (#28388)	undiluted
Cobalt chloride in TBS, pH 7.2	10 mM
EPPS, pH 8.0	100 mM
Ferric chloride in TBS, pH 7.2	10 mM
Glycine	100 mM
Guanidine•HCl	3.5 M
HEPES, pH 7.5	100 mM
Imidazole, pH 7.0	200 mM
MES, pH 6.1	100 mM
MES (0.1 M), NaCl (0.9%), pH 4.7 (#28390)	undiluted
MOPS, pH 7.2	100 mM
Modified Dulbecco's PBS, pH 7.4 (#28374)	undiluted
Nickel chloride in TBS, pH 7.2	10 mM
PBS; Phosphate (0.1 M), NaCl (0.15 M), pH 7.2 (#28372)	undiluted
PIPES, pH 6.8	100 mM
RIPA lysis buffer; 50 mM Tris, 150 mM NaCl, 0.5% DOC, 1% NP-40, 0.1% SDS, pH 8.0	1/10 dilution*
Sodium acetate, pH 4.8	180 mM
Sodium azide	0.5%
Sodium bicarbonate	100 mM
Sodium chloride	5.0 M
Sodium citrate, pH 4.8 or pH 6.4	200 mM
Sodium phosphate	100 mM
Tricine, pH 8.0	100 mM
Triethanolamine, pH 7.8	100 mM
Tris	2.0 M
TBS; Tris (25 mM), NaCl (0.15 M), pH 7.6 (#28376)	undiluted
Tris (25 mM), Glycine (192 mM), pH 8.0 (#28380)	undiluted
Tris (25 mM), Glycine (192 mM), SDS (0.1%), pH 8.3 (#28378)	1/2 dilution*
Zinc chloride in TBS, pH 7.2	10 mM

Substance	Compatible Concentration
Detergents	
Brij®-35	0.125%
Brij®-56, Brij®-58	0.031%
CHAPS, CHAPSO	5.0%
Deoxycholic acid	0.05%
Lubrol® PX	0.125%
Octyl β-glucoside	0.5%
Nonidet P-40 (NP-40)	0.5%
Octyl β-thioglucopyranoside	3.0%
SDS	0.125%
Span® 20	0.5%
Triton® X-100, X-114	0.125%
Triton® X-305, X-405	0.5%
Tween®-20	0.062%
Tween®-60	0.1%
Tween®-80	0.062%
Zwittergent® 3-14	0.025%
Chelating agents	
EDTA	100 mM
EGTA	2 mM
Sodium citrate	200 mM
Reducing & Thiol-Containing Agents	
N-acetylglucosamine in PBS, pH 7.2	100 mM
Ascorbic acid	50 mM
Cysteine	10 mM
Dithioerythritol (DTE)	1 mM
Dithiothreitol (DTT)	5 mM
Glucose	1.0 mM
Melibiose	100 mM
2-Mercaptoethanol	1.0 M
Potassium thiocyanate	3.0 M
Thimerosal	0.01%
Misc. Reagents & Solvents	
Acetone	10%
Acetonitrile	10%
Aprotinin	10 mg/L
DMF, DMSO	10%
Ethanol	10%
Glycerol (Fresh)	10%
Hydrochloric Acid	100 mM
Leupeptin	10 mg/L
Methanol	10%
Phenol Red	0.5 mg/ml
PMSF	1 mM
Sodium Hydroxide	100 mM
Sucrose	10%
TLCK	0.1 mg/L
TPCK	0.1 mg/L
Urea	3.0 M
o-Vanadate (sodium salt), in PBS, pH 7.2	1 mM

*Diluted with ultrapure water.

Messbereich:

a) BCA: 5-250 µg/ml; mit einem modifizierten Protokoll 20-2000 µg/ml
b) Bradford: 0.1-5.0 mg/ml

Vorteile:

a) BCA: Für Detergenz-haltige Lösungen und Membranproteine geeignet. Die Reaktion läuft im alkalischen Milieu ab, in dem fast alle Proteine in Lösung bleiben.
b) Bradford: Farbentwicklung nach 2 min beendet. Variabilität der Färbung zwischen verschiedenen Proteinen ist gering.

Störfaktoren:

a) BCA: Hohe Konzentrationen von Komplex-bildenden Reagenzien (z.B. EDTA), Ammoniumsulfat, N-Acetylglucosamin, Glycin, reduzierende Stoffe wie Glukose, DTT oder Sorbitol und eine Reihe von Pharmaka wie Chlorpromazin, Penicillin und Vitamin C stören.

b) Bradford: Die Probe muss relativ frei von Detergentien sein, da diese bei Konzentrationen über 0,1 % die Bildung des Farbkomplexes behindern. In Gegenwart von Detergentien BCA-Assay verwenden oder Detergenzien durch Dialyse, Ultrafiltration oder Fällung entfernen. Starke Basen stören.

Bei höheren Konzentrationen die Extinktion innerhalb von 15 min bestimmen, da die Proteine in em sauren Milieu leicht präzipitieren.