YamayBio

Ready-to-use BCA Protein Assay Kit

QUICK START GUIDE

Research use only.
Not for diagnostic procedures.

Contents and storage

Ready-to-use BCA Protein Assay Kit

BH5484 500 Assays

Storage: Store at 4°C /-20°C for 12 months. Product shipped in ice packs.

Component	Cat. No.	Volume	Storage
BCA Reagent A	BH5401	100 mL	4°C
BCA Reagent B	BH5402	3 mL	
BSA standards ① 0 μg/mL	BH5411	1 mL	-20°C
BSA standards ② 125 μg/mL	BH5413	1 mL	
BSA standards ③ 250 μg/mL	BH5416	1 mL	
BSA standards ④ 500 μg/mL	BH5419	1 mL	
BSA standards ⑤ 750 μg/mL	BH5420	1 mL	
BSA standards ⑥ 1000 μg/mL	BH5421	1 mL	
BSA standards ⑦ 1500 μg/mL	BH5422	1 mL	
BSA standards ⑧ 2000 μg/mL	BH5423	1 mL	

Introduction

The Ready-to-use BCA Protein Assay Kit is based on the BCA (bicinchoninic acid) method, enabling rapid, stable, and sensitive determination of protein concentration. In an alkaline environment, peptide bonds in proteins reduce Cu^{2+} to Cu^{+}, forming a purple-colored complex with BCA reagents. This complex absorbs light strongly at 562 nm, and the absorbance is directly proportional to the protein concentration.

The kit is compatible with a wide range (**20 to 2000 μg/mL**) of samples and is tolerant to common interfering substances such as metal ions, reducing agents, chelating agents, and detergents. It also includes pre-diluted BSA standards, eliminating the need for labor-intensive dilution steps and improving accuracy and convenience.

Quick Protocol

1. Mix Reagents A and B 50:1 to create the Working Reagent.
2. **20 µL** sample or standards + **200 µL** of Working Reagent.
3. Incubate for 30 minutes at 37°C.
4. Read at 562nm.

Prepare BCA working reagent (WR)

1. Use the following formula to calculate the total volume of WR required for the assay: Total volume of WR = (# standards + # samples) × (# replicates) × (volume of WR per sample)
   Example: 8 standards, 3 samples and 3 replicates : (8 standards + 3 unknowns) × (3 replicates) × (200µL) = 6.6 mL WR
2. According to the calculated amount of the required working reagent, mix the reagent A and reagent B at 50:1 volume ratio.

Note:

1. Due to the accidental error in sample addition, it is recommended to prepare extra 1-2 samples of BCA working solution to ensure a sufficient volume of WR.
2. The newly prepared BCA working solution can be stored stably for 24 hours at room temperature under sealed conditions.

Detail Procedures for BCA Protein Assay (Microplate)

1. Add **20 µL** of ready-to-use BSA standards (① to ⑧) to a 96-well microplate.
   Note: Ensure BSA standards are fully dissolved and thoroughly mixed before use.
2. Add **20 µL** of each sample (in replicate) into designated wells. (Working range: 20–2000 µg/mL)
   Note: It is strongly recommended to dilute samples with 1× PBS or 0.9% saline to ensure they fall within the working range.
3. Add 200 µL of WR to each well. Mix thoroughly using a plate shaker for 30 seconds. Cover the plate and incubate at 37 °C for 30 minutes.
4. Allow the plate to equilibrate to room temperature. Measure absorbance at 562 nm using a microplate reader.
5. Subtract the average 562 nm absorbance of the blank standard replicates from the readings of all other standards and unknown samples.
6. Prepare a standard curve by plotting the average blank-corrected absorbance at 562 nm for each BSA standard against its concentration (µg/mL). Use this curve to determine the protein concentration of unknown samples.

Note:

1. If using curve-fitting algorithms associated with a microplate reader, a four-parameter (quadratic) or best–fit curve provides more accurate results than a purely linear fit.
2. Data processing requires the removal of obviously incorrect values.

Compatible substance concentrations

Salts/Buffers	Compatible Concentration
Ammonium Sulfate	disturb
Sodium Chloride	1M
Urea	3M
Acetate	0.2M
Glycine	1M
HEPES	0.1M
MES	50mM
MOPS	50mM
Sodium Citrate	<1mM
PIPES	50mM
Sodium Phosphate	0.1M
Sodium Acetate	0.2M, pH 5.5
TES	50mM
Tris	0.1M
Polar compound	**Compatible Concentration**
DMSO	5%
Glycerinum	10%
Chelating Agent	**Compatible Concentration**
EDTA	10mM

Detergent	Compatible Concentration
Brij35	1%
CHAPS	1%
Guanidine Hydrochloride	4M
NP-40	1%
Siminous Glucose	1%
SDS	1%
Triton X-100	1%
Saccharides	**Compatible Concentration**
Glucose	10mM
Saccharose	1M
Reducing Agent	**Compatible Concentration**
beta-mercaptoethanol	50μM
DTT	1mM
Other	**Compatible Concentration**
HCl/NaOH	0.1M
Lipid	disturb