ARBOR ASSAYS™
Interactive Assay Solutions™

MULTI SPECIES

MOST SENSITIVE

DetectX®

BCA Protein
Colorimetric Detection Kit

2 Plate Kit Catalog Number K041-H1

Species Independent

DUAL RANGE

Sample Types Validated:

Serum, Plasma, Tissue Homogenates and Cell Lysates

Please read this insert completely prior to using the product.
For research use only. Not for use in diagnostic procedures.
Not for human diagnostic use.

www.ArborAssays.com

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BACKGROUND

Protein determination is one of the most common operations performed in biochemical research. The principle of the bicinchoninic acid (BCA) assay is similar to the Lowry assay[1], and relies on the formation of a Cu^{2+}-protein complex under alkaline conditions[2], followed by reduction of the Cu^{2+} to Cu^{1+}. The amount of reduction is proportional to protein present. It has been shown that cysteine, cystine, tryptophan, tyrosine, and peptide bonds[2] are able to reduce Cu^{2+} to Cu^{1+}. BCA forms a purple-blue complex with Cu^{1+} in alkaline environments, thus providing a basis to monitor the reduction of alkaline Cu^{2+} by proteins.

The method combines the well-known reduction of Cu^{2+} to Cu^{1+} by protein in an alkaline medium (the biuret reaction) with the highly sensitive and selective colorimetric detection of the cuprous cation (Cu^{+1}) using a unique reagent containing bicinchoninic acid. The purple-colored reaction product of this assay is formed by the chelation of two molecules of BCA with one cuprous ion. This water-soluble complex exhibits a strong absorbance at 562nm that is nearly linear with increasing protein concentrations over a broad working range (6-1,000 µg/mL). The BCA chemistry is not a true end-point method; that is, the final color continues to develop. However, following incubation, the rate of continued color development is sufficiently slow to allow large numbers of samples to be assayed together.

1. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ., “Protein measurement with the Folin phenol reagent”. J. Biol. Chem. 1951, 193 (1): 265–75.
2. Smith, PK, et. al., “Measurement of protein using bicinchoninic acid.”, Anal. Biochem., 1985, 150: (1), 76-85.

ASSAY PRINCIPLE

The DetectX® BCA Protein Assay Kit is designed to quantitatively measure total protein content in a variety of samples. The assay measures all types of proteins from all species. Please read the complete kit insert before performing this assay. A bovine serum albumin (BSA) standard is provided to generate a standard curve for the assay and all samples should be read off of the standard curve. Samples are diluted in water and added to the wells. The BCA Color Solution is made by mixing the BCA Reagent with the BCA Enhancer. The BCA Color Solution is added to all wells and the plate incubated at 37°C. Protein in the samples reacts with the BCA Color Reagent to generate a purple colored product which is read at 560 nm.

RELATED PRODUCTS

Kits	Catalog No.
Cyclic AMP Direct ELISA Kits	K019-H1/H5
Cyclic AMP Direct Chemiluminescent ELISA Kits	K019-C1/C5
Cyclic GMP Direct ELISA Kits	K020-H1/H5
Cyclic GMP Direct Chemiluminescent ELISA Kits	K020-C1/C5
Cyclic GMP Direct ELISA Kits – Improved Sensitivity	K065-H1/H5
Hemoglobin Colorimetric Detection Kit	K013-H1
Hemoglobin High Sensitivity Colorimetric Detection Kits	K013-HX1/HX5
Protein Kinase A (PKA) Activity Kit	K027-H1
Cell Lysis Buffer	X050-100ML

SUPPLIED COMPONENTS

Clear Half Area 96 Well Plates
Corning Costar Plate 3695.

2 Plates	Catalog Number X018-2EA

Bovine Serum Albumin Standard
Bovine Serum Albumin (BSA) at 10 mg/mL.

200 µL	Catalog Number C143-200UL

BCA Reagent
BCA Reagent solution.

16 mL	Catalog Number C144-16ML

BCA Enhancer
BCA Enhancer solution containing copper sulfate.

320 µL	Catalog Number C145-320UL

Plate Sealers

2 Each	Catalog Number X002-1EA

STORAGE INSTRUCTIONS

All components of this kit should be stored at room temperature until the expiration date of the kit.

OTHER MATERIALS REQUIRED

Deionized water.

Repeater pipet with disposable tips capable of dispensing 75 µL.

96 well plate reader capable of reading optical absorption at 560 nm. Set plate parameters for a 96-well Corning Costar 3695 plate. See: www.ArborAssays.com/resources/#general-info for plate dimension data.

Software for converting optical density (OD) readings from the plate reader and carrying out four parameter logistic curve (4PLC) fitting. Contact your plate reader manufacturer for details.

PRECAUTIONS

As with all such products, this kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

The Bovine Serum Albumin (BSA) standard contains low concentrations (≤0.9%) of sodium azide. Please dispose of any solutions using this material with copious amounts of water.

SAMPLE TYPES AND PREPARATION

Samples should be diluted in deionized water. Two protocols are listed to allow most samples to be read and samples with very low levels of protein to be measured. Dilutions should be made to ensure that protein levels for samples fall within the standard curve range.

Cell lysates in our Cell Lysis Buffer, X050-100ML, should be diluted 1:10 or greater with deionized water to bring the total protein content in the analyzed sample down to 1 mg/mL or lower.

Human serum and plasma samples will typically have between 60 and 100 mg of total protein per mL. They should be diluted 1:100 or greater in deionized water to be measured in this kit.

Most urine samples will have levels between 50 to 200 µg/mL. Urine samples should be diluted at least 1:2 with deionized water prior to testing.

SAMPLE INCOMPATIBILITIES

The assay will tolerate most common laboratory chemicals. Known incompatibilities include reducing agents such as dithioerythritol (DTE), dithiothreitol (DTT) and β-mercaptoethanol (BME) at concentrations above 1 mM. Organic solvent content should be below 1%.

SAMPLE COMPATIBILITIES

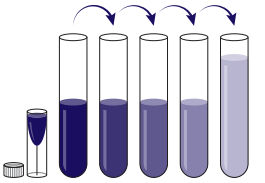
Sample	Compatibility
Most buffers + other salts	< 10 mM
Most detergents (Triton, Tween, SDS, Nonidet, etc.)	< 1%
Ammonium Sulfate	≤ 1M
EDTA + EGTA	< 2 mM
Glycerol	≤ 10%
Guanidine Hydrochloride	≤ 3M
Urea	≤ 3M

A simple dilution into water should bring most samples down to the appropriate level.

STANDARD PREPARATION - REGULAR FORMAT

Standard Preparation

BSA Standards are prepared by labeling tubes as #1 through #6. Add 180 µL of water to tube #1 and pipet 100 µL of water into tubes #2 to #6. Carefully add 20 µL of the BSA Standard Stock to tube #1 and vortex. Add 100 µL of tube #1 to tube #2 and vortex completely. Repeat this for tubes #3 through #6. The concentration of BSA in tubes 1 through 6 will be 1,000, 500, 250, 125, 62.5, and 31.25 µg/mL.

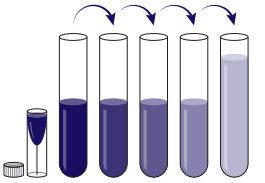


	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Deionized Water (µL)	**180**	100	100	100	100	100
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5
Volume of Addition (µL)	**20**	100	100	100	100	100
Final Conc (µg/mL)	1,000	500	250	125	62.5	31.25

STANDARD PREPARATION - HIGH SENSITIVITY

Standard Preparation

BSA Standards are prepared by labeling tubes as #1 through #6. Add 490 µL of water to tube #1 and pipet 200 µL of water into tubes #2 to #6. Carefully add 10 µL of the BSA Standard Stock to tube #1 and vortex. Add 200 µL of tube #1 to tube #2 and vortex completely. Repeat this for tubes #3 through #6. The concentration of BSA in tubes 1 through 6 will be 200, 100, 50, 25, 12.5, and 6.25 µg/mL.



	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Deionized Water (µL)	**490**	200	200	200	200	200
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5
Volume of Addition (µL)	**10**	200	200	200	200	200
Final Conc (µg/mL)	200	100	50	25	12.5	6.25

Use all Standards within 2 hours of preparation.

BCA Color Solution Preparation

Measure out the BCA Reagent into a clean container. Add BCA Enhancer to the container and vortex. Solution will turn light green in color.

	1/2 Plate	1 Plate	1.5 Plates	2 Plates
BCA Reagent	3.92 mL	7.84 mL	11.76 mL	14.7 mL
BCA Enhancer	80 µL	160 µL	240 µL	300 µL
BCA Color Solution Final Volume	4 mL	8 mL	12 mL	15 mL

Prepared BCA Color Solution should be used within 12 hours.

We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine sample concentrations.

ASSAY PROTOCOL - REGULAR SENSITIVITY

Use the plate layout sheet on the back page to aid in proper sample and standard identification.

1. Pipet 10 µL of samples or appropriate standards into duplicate wells in the plate.
2. Pipet 10 µL of water into duplicate wells as the Zero standard.
3. Add 75 µL of the BCA Color Solution to each well using a repeater pipet.
4. Seal the plate and incubate at 37°C for 2 hours.
5. Read the optical density at 560 nm.

ASSAY PROTOCOL - HIGH SENSITIVITY

Use the plate layout sheet on the back page to aid in proper sample and standard identification.

1. Pipet 50 µL of samples or appropriate standards into duplicate wells in the plate.
2. Pipet 50 µL of water into duplicate wells as the Zero standard.
3. Add 75 µL of the BCA Color Solution to each well using a repeater pipet.
4. Seal the plate and incubate at 37°C for 2 hours.
5. Read the optical density at 560 nm.

CALCULATION OF RESULTS

Average the duplicate OD readings for each standard and sample. Create a standard curve by reducing the data using computer software capable of generating a four-parameter logistic curve (4PLC) fit. The sample concentrations obtained should be multiplied by the dilution factor to obtain neat sample values.

Or use the online tool from MyAssays to calculate the data:

Regular:
https://www.myassays.com/arbor-assays-bca-protein-colorimetric-detection-kit-low-range-k041.assay

High Sensitivity:
https://www.myassays.com/arbor-assays-bca-protein-colorimetric-detection-kit-high-range-k041.assay

TYPICAL DATA - REGULAR FORMAT

Sample	Mean OD	Protein Concentration (µg/mL)
Standard 1	1.618	1,000
Standard 2	0.914	500
Standard 3	0.516	250
Standard 4	0.320	125
Standard 5	0.194	62.5
Standard 6	0.138	31.25
Zero	0.088	0
Sample 1	1.119	638.2
Sample 2	0.313	127.1

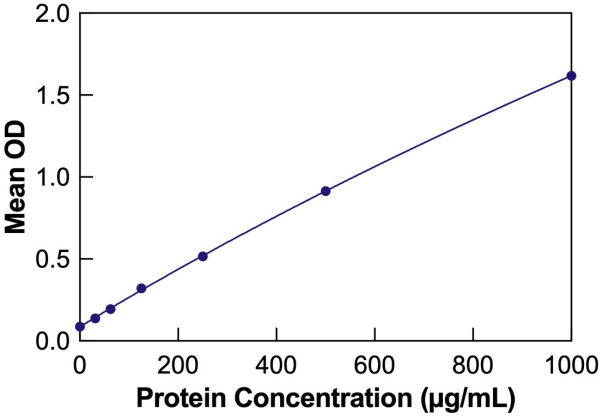
TYPICAL DATA - HIGH SENSITIVITY FORMAT

Sample	Mean OD	Protein Concentration (µg/mL)
Standard 1	1.605	200
Standard 2	0.960	100
Standard 3	0.554	50
Standard 4	0.365	25
Standard 5	0.211	12.5
Standard 6	0.160	6.25
Zero	0.095	0
Sample 1	1.009	107.1
Sample 2	0.358	26.5

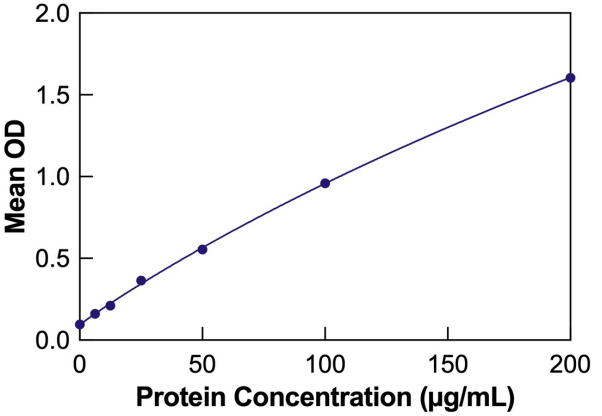
Always run your own standard curves for calculation of results. Do not use this data.

TYPICAL STANDARD CURVES

Regular Format



High Sensitivity Format



Always run your own standard curves for calculation of results. Do not use this data.

VALIDATION DATA

Sensitivity and Limit of Detection - Regular Format

Sensitivity was determined by comparing the ODs for twenty wells run for each of the zero and standard #6. The detection limit was determined at two (2) standard deviations from the zero along the standard curve. **Sensitivity was determined as 6.65 µg/mL.**

Limit of Detection was determined in a similar manner by comparing the OD's for twenty runs for each of the zero standard and a low concentration serum sample. **Limit of Detection was determined as 6.98 µg/mL.**

Sensitivity and Limit of Detection - High Sensitivity Format

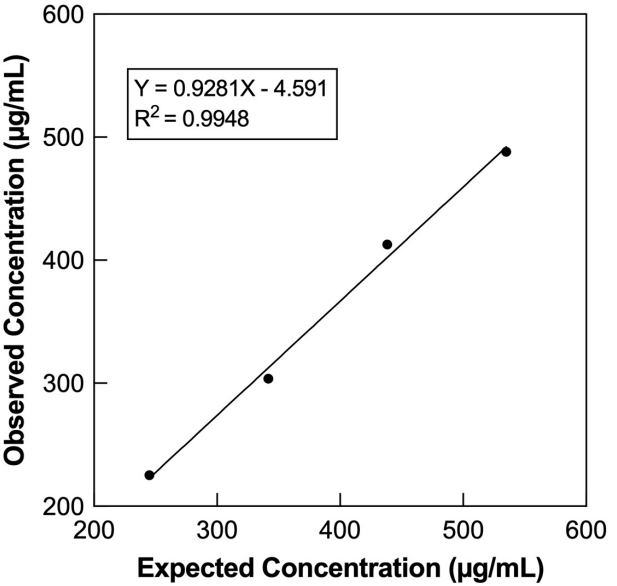
Sensitivity was determined by comparing the ODs for twenty wells run for each of the zero and standard #6. The detection limit was determined at two (2) standard deviations from the zero along the standard curve. **Sensitivity was determined as 1.68 µg/mL.**

Limit of Detection was determined in a similar manner by comparing the OD's for twenty runs for each of the zero standard and a low concentration urine sample. **Limit of Detection was determined as 2.68 µg/mL.**

Linearity

Linearity was determined in the Regular Format by taking two human samples, one with a known high total protein concentration and another with a lower total protein concentration and mixing them in the ratios given below. The measured concentrations were compared to the expected values based on the ratios used.

High Sample	Low Sample	Expected Conc. (ug/mL)	Observed Conc. (ug/mL)	% Recovery
80%	20%	535.1	488.1	91.2
60%	40%	438.4	412.7	94.1
40%	60%	341.7	303.6	88.9
20%	80%	245.0	225.3	91.9
			Mean Recovery	**91.5%**



Intra Assay Precision - Regular Format

Three samples diluted in water were run in replicates of 20 in an assay. The mean and precision of the calculated concentrations were:

Sample	Protein Conc. (µg/mL)	%CV
1	584.9	3.5
2	285.5	6.9
3	118.4	5.5

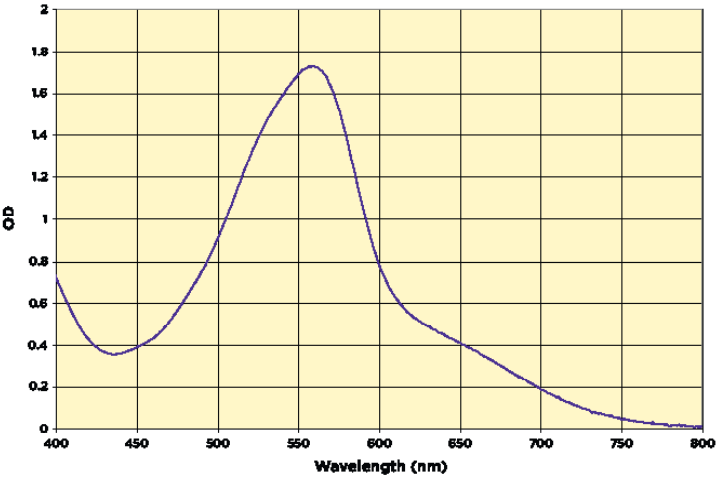
Inter Assay Precision - Regular Format

Three samples diluted in water were run in duplicates in thirteen assays run over multiple days by three operators. The mean and precision of the calculated concentrations were:

Sample	Protein Conc. (µg/mL)	%CV
1	631.1	3.8
2	297.7	6.3
3	119.6	10.4

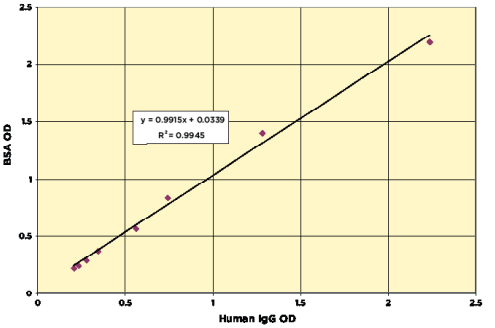
Color Spectrum

The spectra of the BCA-protein purple colored reaction product is shown below.



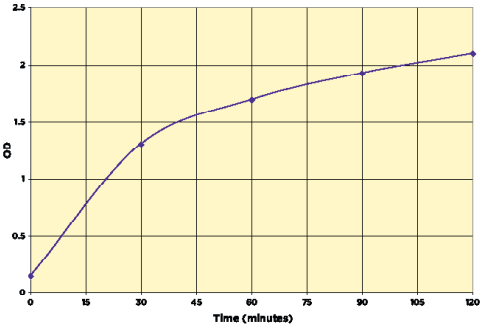
PROTEIN COMPARISON

Standards made with BSA and human IgG were compared.

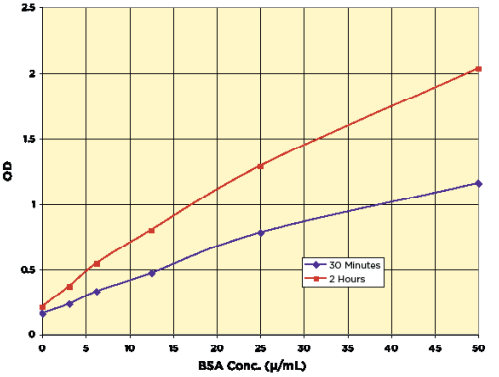


KINETICS OF COLOR DEVELOPMENT

The BCA assay chemistry is not a true end-point method. The final color continues to develop however the rate of continued color development is relatively slow after an hour at 37°C to allow large numbers of samples to be assayed together.



There will be lower signals at shorter incubations, however adequate and sensitive standard curves can be developed even with 30 minute assay formats.



LIMITED WARRANTY

Arbor Assays warrants that at the time of shipment this product is free from defects in materials and workmanship. This warranty is in lieu of any other warranty expressed or implied, including but not limited to, any implied warranty of merchantability or fitness for a particular purpose.

We must be notified of any breach of this warranty within 48 hours of receipt of the product. No claim shall be honored if we are not notified within this time period, or if the product has been stored in any way other than outlined in this publication. The sole and exclusive remedy of the customer for any liability based upon this warranty is limited to the replacement of the product, or refund of the invoice price of the goods.

CONTACT INFORMATION

For details concerning this kit or to order any of our products please contact us:

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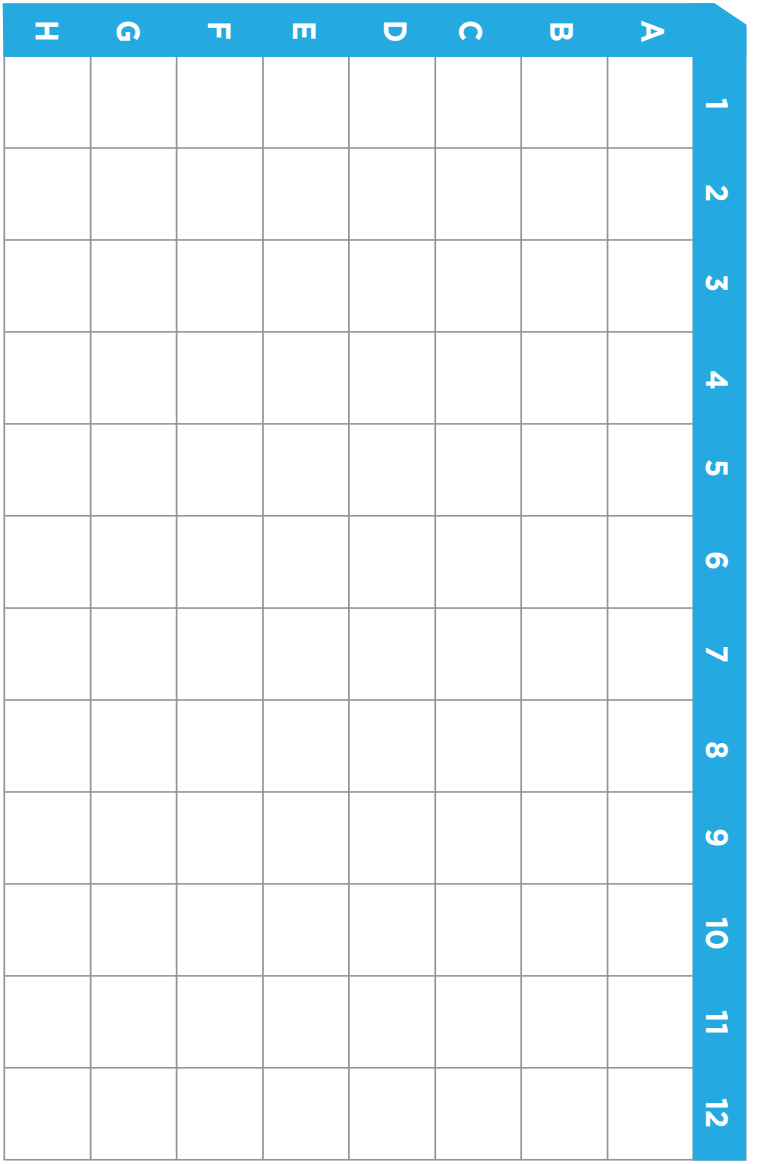
OFFICIAL SUPPLIER TO ISWE

Arbor Assays and the International Society of Wildlife Endocrinology (ISWE) signed an exclusive agreement for Arbor Assays to supply ISWE members with assay kits and reagents for wildlife conservation research.



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