Bromophenol Blue Protein Assay Kit

Catalog # 6026

For Research Use Only - Not Human or Therapeutic Use

PRODUCT SPECIFICATIONS

DESCRIPTION:	Assay kit to quantify proteins (proteinuria)
FORMAT:	96-well ELISA Plate with removeable strips
ASSAY TYPE:	Colorimetric Assay
ASSAY TIME:	5 minutes
STANDARD RANGE:	2 mg/ml to 0.03 mg/ml
NUMBER OF SAMPLES:	Up to 40 (duplicate) samples/plate
SAMPLE TYPES:	Urine, Serum, and Plasma
RECOMMENDED SAMPLE DILUTIONS:	Varies
CHROMOGEN:	N/A (read at 610 nm)
STORAGE:	4°C
VALIDATION DATA:	Intra-Assay (1.7-5.2%)/Inter-Assay (4.4-9.8%)/Spiking Test (93-105%)
NOTES:	

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INTRODUCTION

Bromophenol blue (BPB), a phenolphthalein anionic dye, binds to proteins under neutral to acidic conditions. From this attribute, BPB can determine protein levels in samples, especially in solubilized cultured cells and cell membrane proteins with high concentrations of surfactants (8). This is an advantage over the widely used Coomassie blue protein assay which is affected by the presence of surfactants in the samples.

Proteinuria, the presence of an excess of serum proteins in the urine, is a useful marker of renal disease. In addition, the pathophysiology of proteinuria can be divided into tubular or glomerular dysfunction. For example, albumin, a plasma protein, may better define pathological proteinuria of glomerular origin while beta 2-microglobulin indicates tubular origin (6, 7). Usually, urinary protein levels are determined using a dipstick assay. However, this method is affected by urine volume and color, which can lead to inaccurate results (1-3). Therefore, as a simple, precise, and accurate alternative for quantifying proteinuria, a BPB protein assay is available. The results may better reflect glomerular albuminuria due to its higher affinity for albumin rather than globulin (4, 5).

Chondrex, Inc. provides a BPB Protein Assay Kit (Cat # 6026) to evaluate proteinuria in mice and rats, as well as a Rat Urinary Protein Assay Kit (Catalog # 9040), a Rat Albumin Detection ELISA Kit (Catalog # 3020), and a Mouse Albumin Detection ELISA Kit (Catalog # 3012).

KIT COMPONENTS

Item	Quantity	Amount	Storage
Bovine Serum Albumin Standard (60261)	1 vial	2 mg, Lyophilized	4°C
Solution A (60262)	1 bottle	18 ml	4°C
Solution B (60263)	1 bottle	6 ml	4°C
PBS (60264)	1 bottle	50 ml	4°C
ELISA Plate	1 each	96-well (8-well strips x 12)	4°C

ASSAY OUTLINE

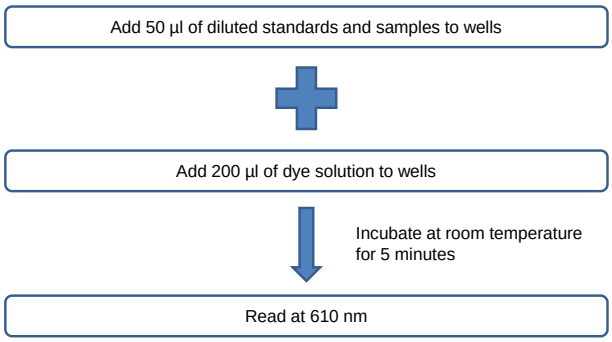

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PLATE LAYOUT

	1	2	3	4	5	6	7	8	9	10	11	12
A	2	2	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33
B	1	1	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34
C	0.5	0.5	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35
D	0.25	0.25	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36
E	0.125	0.125	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37
F	0.63	0.63	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38
G	0.31	0.31	S7	S7	S15	S15	S23	S23	S31	S31	S39	S39
H	B	B	S8	S8	S16	S16	S24	S24	S32	S32	S40	S40

Standards | Samples

NOTES BEFORE USING ASSAY

NOTE 1: It is recommended that the standard and samples be run in duplicate.

NOTE 2: Warm up all buffers to room temperature before use.

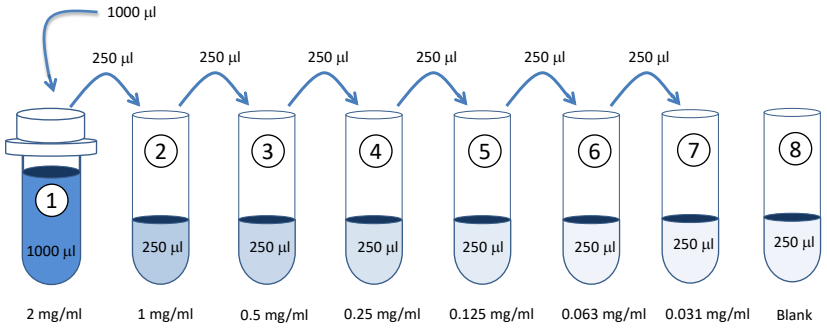
NOTE 3: Crystals may form in Wash Buffer, 20X when stored at cold temperatures. If crystals have formed, warm the wash buffer by placing the bottle in warm water until crystals are completely dissolved.

NOTE 4: Measure exact volume of buffers using a serological pipet, as extra buffer is provided.

NOTE 5: This kit contains animal components from non-infectious animals and should be treated as potential biohazards in use and for disposal.

ASSAY PROCEDURE

1. **Prepare Standard Dilutions**: The recommended standard range is 0.031 - 2 mg/ml. Dissolve one vial of Standard in 1 ml of PBS for the 2 mg/ml standard and serially dilute with PBS. For example, mix 200 µl of the standard (2 mg/ml) with an equal volume of PBS to make a 1 mg/ml solution, and then repeat it five more times for 0.5, 0.25, 0.125, 0.063, and 0.031 mg/ml solutions. The remaining 2 mg/ml standard stock may be stored at 4°C for future use. Chondrex, Inc. recommends making fresh serial dilutions for each assay.



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2. **Prepare Sample Dilutions**: Dilute samples with PBS. It is recommended to use 2-3 different dilutions if the sample albumin level is unknown.

3. **Prepare Dye Solution**: Mix 150 µl of Solution A with 50 µl of Solution B for each well just before use. For example, 8 samples, 7-point standards, and one blank (all in duplicate) will require 6.4 ml of the Dye Solution. Mix 4.8 ml of Solution A with 1.6 ml of Solution B.

4. **Add Standards and Samples**: Add 50 µl of standards, PBS (Blank), and samples to designated wells.

5. **Add Dye Solution**: Add 200 µl of the dye solution into all wells. Incubate at room temperature for 5 minutes.

6. **Read Plate**: Read the OD values at 610 nm. If the OD values of samples are greater than the OD values of the highest standard, re-assay the samples at a higher dilution.

CALCULATING RESULTS

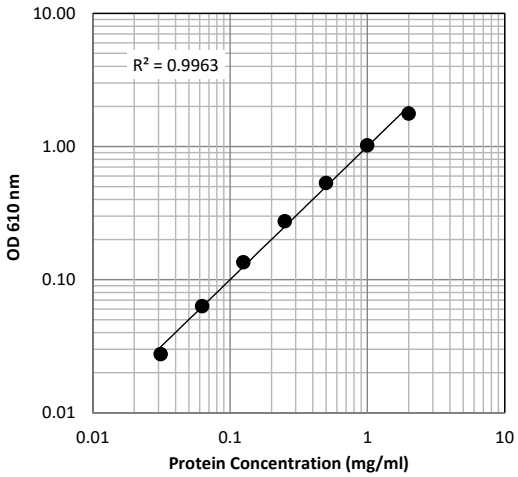
1. Average the duplicate OD values for the standards, blank (B), and test samples.

2. Subtract the "blank" (B) values from the averaged OD values of the standards and test samples.

3. Plot the OD values of standards against the mg/ml of standard. Using a log/log plot will linearize the data. Figure 1 shows a representative experiment where the standard range is from 0.031-2 mg/ml.

4. The mg/ml of protein in test samples can be calculated using regression analysis. Multiply it by the sample dilution factor to obtain the protein concentration (mg/ml) in original test samples. For additional assistance, please download a sample calculation worksheet from www.chondrex.com.

Figure 1 - A Typical Standard Curve for the BPB Assay Kit



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VALIDATION DATA

Table 1 - Reproducibility Data for the BPB Assay Kit

Test At	0.07 mg/ml	0.3 mg/ml	1 mg/ml
Intra-Assay CV (%)	1.7	5.2	3.3
Inter-Assay CV (%)	9.8	6.2	4.4
Spiking Test*	105%	101 %	93 %

*Standard was added with known amounts of albumin and then diluted with PBS to assay the protein concentration.

TROUBLESHOOTING

For frequently asked questions about assays and ELISAs, please see Chondrex, Inc.'s Assay FAQ for more information.

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