Chondrex, Inc.

Bromophenol Blue Protein Assay Kit

Catalog # 6026

For Research Use Only - Not Human or Therapeutic Use

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.

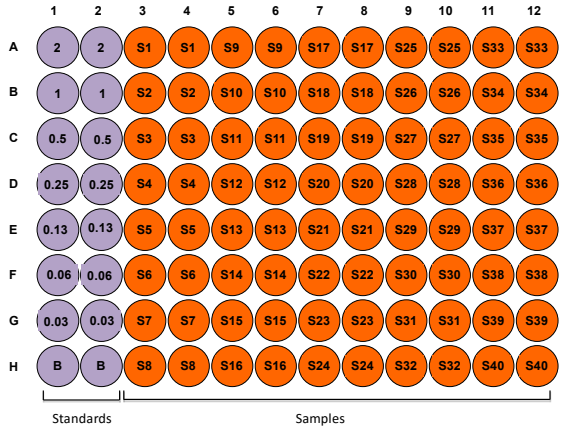
Proteinuria, the presence of an excess of serum proteins in the urine, is a useful marker of renal disease. In addition, the pathophysiology of proteinuria can be divided into tubular or glomerular dysfunction. For example, albumin, a plasma protein, may better define pathological proteinuria of glomerular 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 (Catalog # 6026) to evaluate proteinuria in mice and rats, as well as a Rat Urinary Protein Assay Kit (Catalog # 9040), a Rat Albumin Assay Kit (Catalog # 3020), and a Mouse Albumin Assay 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

Figure 1 - A Standard Assay Layout



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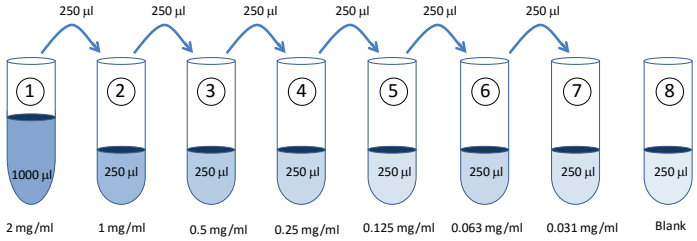
ASSAY PROCEDURE

1. **Prepare Standard Dilutions**: The recommended standard range is 0.031 - 2 mg/ml. Dissolve one vial of Albumin Standard in 1 ml of PBS for the 2 mg/ml standard. Then serially dilute it 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 -20°C for use in a second assay. We recommend making fresh serial dilutions for each assay.



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**: Use the plate layout as shown in Figure 1. 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.

CALCULATION OF PROTEIN CONCENTRATION

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 2 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 Chondrex. Inc.'s web site.

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Figure 2 - A typical standard curve for BPB protein assay

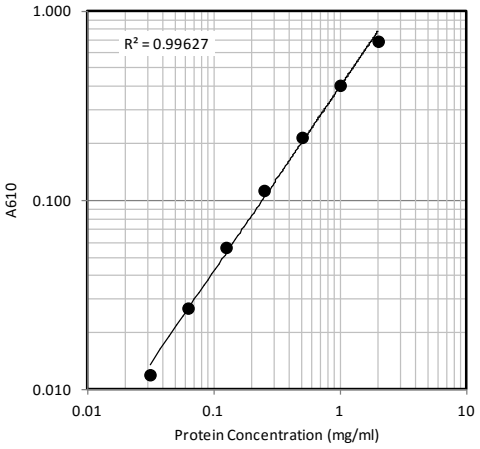

Table 1 - Reproducibility of data assayed by BPB Protein Assay Kit

Test At	1 mg/ml	0.3 mg/ml	0.07 mg/ml
Inter-Assay CV (%)	4.4	6.2	9.8
Intra-Assay CV (%)	3.3	5.2	1.7
Spiking Test*	92.5 %	100.2 %	104.3%

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

REFERENCES

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