# QuantiChrom™ Total Protein Assay Kit

## Quantitative Colorimetric Determination of Total Protein

### DESCRIPTION

Total protein concentration can be determined by several methods. However, protein dye-binding procedures are simple, reliable, rapid, readily automated and are becoming increasingly popular in assessing renal and blood brain barrier functions.

BioAssay Systems' Total Protein Assay Kit is based on an improved pyrogallol red-molybdate protein dye-binding assay. The color intensity at 600nm is directly proportional to the total protein concentration in the sample. This single reagent can be used for the quantitative determination of total protein in urine (proteinuria) and cerebrospinal fluid (blood brain barrier).

### KEY FEATURES

*Fast and sensitive.* Assay takes 10 min. Linear detection range of 5 - 200 mg/dL protein. For urine samples, 1 - 20 mg/dL protein detection range.

Robust and Reliable. Substances in urine have minimal interference (< 2%).

*Convenient and high-throughput.* Homogeneous "mix-incubate-measure" type assay. Can be readily automated on HTS liquid handling systems for processing thousands of samples per day.

### APPLICATIONS

Determination of total protein in biological samples (e.g. urine, CSF).

### KIT CONTENTS

| Catalog# | Reagent | Standard | Tests (96-well) | Tests (Cuvette) |
|---|---|---|---|---|
| QTPR-100 | 20 mL | 1 mL 100 mg/dL | 100 | 20 |
| QTPR-01K | 200 mL | 1 mL 100 mg/dL | 1000 | 200 |
| For bulk orders of > 1000 mL Reagent, please inquire for a quote. | | | | |

***Storage conditions***. This product is shipped at room temperature. Store kit at 2-8°C. Shelf life of 12 months after receipt.

***Precautions:*** Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

### ASSAY PROCEDURE FOR 96-WELL PLATE READER

Prior to assay, bring all reagents to room temperature.

1. *Standards and Samples.* Pipette 5 µL $dH_2O$, 5 µL provided Protein Standard and 5 µL Samples into separate wells of a clear flat-bottom 96-well plate.
2. *Assay*. Add 200 µL Reagent to all assay wells. Tap plate to mix. Incubate for 10 min. Measure $OD_{600nm}$ on a plate reader.

***Urine Samples***

Urine samples typically have very low protein concentrations and require a larger sample volume and an internal standard. The following procedure should be used:

1. In a centrifuge tube, dilute the 100 mg/dL Standard to 40 mg/dL in $dH_2O$ (40 µL 100 mg/dL Standard + 60 µL $dH_2O$).
2. Samples are run in duplicate. If particulates are present, centrifuge the sample and use the clear supernatant for the assay. Transfer 20 µL of each sample into four separate wells: two Sample wells and two Internal Standard wells.

   Add 5 µL $dH_2O$ to Sample wells, and 5 µL of the 40 mg/dL Standard to the Internal Standard wells.

   Transfer 25 µL of $dH_2O$ into two wells. This will be the Blank in duplicate. Note: Each sample does not require a separate Blank, the same Blank value can be used for all samples on a particular plate.
3. Add 200 µL Reagent to each protein determination wells.
4. Incubate 10 min at room temperature, and then read the optical density at 600 nm.

*Note:* if the $OD_{STANDARD}$ - $OD_{SAMPLE}$ for a particular sample is lower than 0.05, dilute sample with an equal volume of water and repeat the assay. Multiply result by the dilution factor 2. A low internal standard signal is due to interference with other molecules in urine, dilution will decrease the interference allowing for proper measurement of protein level.

### ASSAY PROCEDURE FOR CUVETTE

1. *Standards and Samples.* Pipette 20 µL $dH_2O$, 20 µL provided Protein Standard and 20 µL Samples into separate cuvettes.
2. *Assay*. Add 1 mL Reagent to each cuvette. Mix and incubate for 10 min. Measure $OD_{600nm}$ on a spectrophotometer.
3. Incubate 10 min at room temperature, and then read the optical density at 600 nm.

### CALCULATION

Total protein concentration of a Sample is calculated as

$$[\text{Protein}] = \frac{OD_{SAMPLE} - OD_{H2O}}{OD_{STANDARD} - OD_{H2O}} \times 100 \times n \quad (mg/dL)$$

where $OD_{SAMPLE}$, $OD_{STANDARD}$ and $OD_{H2O}$ are the optical density values of the Sample, the Standard and the $H_2O$ (blank), respectively. If calculated protein concentration is higher than 200 mg/dL dilute Sample in water and repeat assay. Multiply the results by the dilution factor *n*.

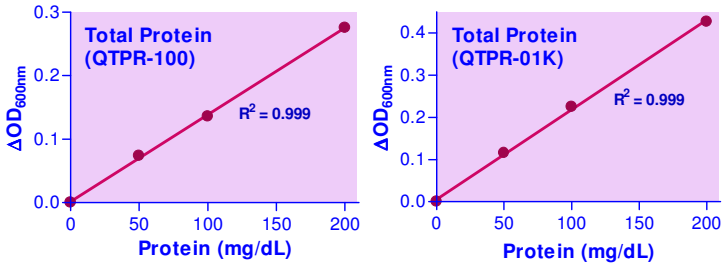
For Samples using an internal standard (e.g. urine) Total protein concentration of a Sample is calculated as

$$[\text{Protein}] = \frac{OD_{SAMPLE} - OD_{BLANK}}{OD_{STANDARD} - OD_{SAMPLE}} \times 10 \times n \quad (mg/dL)$$

where $OD_{SAMPLE}$, $OD_{STANDARD}$, and $OD_{BLANK}$ are the optical density values of the Sample, Internal Standard, and Blank wells, respectively. 10 mg/dL (the volume of the internal standard is ¼ that of the sample) is the concentration of the protein Internal Standard and *n* is the dilution factor

### MATERIAL REQUIRED BUT NOT PROVIDED

For 96-well plate assays: pipetting devices, clear flat bottom 96-well plates, and plate reader. For cuvette assays: pipetting devices, cuvettes and spectrophotometer capable of reading $OD_{600nm}$.




*Left*: Standard curve performed on a 96-well plate reader (Spectramax M2); *Right*: Standard curve using the cuvette procedure.

### LITERATURE

1. Joern WA, Schmoele L (1981). Urinary protein measurement by the Coomassie blue dye-binding method adapted to the ABA-l00 bichromatic analyzer. Clin Chem27:1305.
2. Sano K, et al (1981). Automatic assay of urinary protein using CoomassieBrilliant Blue G-250. Anal Biochem 113:197-201.
3. Watanabe N, et al (1984). Automatic method for determination of protein in urine with a Hitachi 726 analyzer: a modification of Bradford's method. Anal Chem Specimen 7:27-31.