ITSI BIOSCIENCES

TOTAL PROTEIN ASSAY USING THE *ITSIPREP*™ ToPA KIT

IMPORTANT: K-0014-96 is a validated kit and procedure developed for scientists who desire to accurately measure the total protein concentration of samples in a high throughput format using the distinctive ToPA microtiter protein assay kit. The *ITSIPREP*™ Quanti-Protein Assay Reagent is compatible with many procedures and can tolerate many common laboratory buffers. ToPA can be used to quantify proteins isolated from microorganisms, cell lines, whole tissue, blood, serum and plasma. The easy-to-follow procedure and Ready-to-Use reagents makes protein quantitation easy and reproducible.

Read the procedure completely and assemble all materials needed before starting.

MATERIALS PROVIDED IN THIS KIT (Sufficient for 96 Assays):

Item	Size	Catalog #	Storage
Quanti - Protein Assay Reagent (Q-PAR)	1 x 50mL	Cat #: K-0014-96.1	4° C
Standard Curve Reagents (SCR)	7 x 1.0mL	Cat #: K-0014-96.2	Rm. T.
2000 ug/mL Standard Curve Reagent (SCR)	1 x 1.0mL	Cat #: K-0014-96.3	Rm. T.
Solubilization Buffer	1 x 5.0mL	Cat #: K-0014-96.4	-20° C
Microtiter Plate	1 x 96 wells	Cat #: K-0014-96.5	Rm. T.
Procedure			

MATERIALS REQUIRED BUT NOT SUPPLIED:

1. Vortex mixer
2. Adjustable pipettes (a multi channel pipette should be used if available)
3. Microtiter Plate Reader

PROCEDURE:

A. Preparation of the Standard Curve:

1. Before beginning, bring the Quanti-Assay Reagent up to room temperature.
2. Vortex the Standard Curve Reagents tubes well to ensure they are completely mixed.
3. To prepare a standard curve, pipette 10uL of each **standard** into a microtiter well on the microtiter plate. The standards should be prepared in duplicate or triplicate.
4. Mix the Quanti-Protein Assay Reagent well by inverting the bottle 3-5 times (do not shake vigorously), and add 300uL of **Quanti-Protein Assay Reagent** to each standard.
5. Carefully mix by taping the microtiter plate with one hand while holding with the other. Alternatively, a microtiter plate mixer can be used for mixing (Note: Use the slowest speed possible to avoid spillage and cross contamination of samples).
6. Zero the plate reader at 595nm with clean distilled water. If 595nm is not possible use any wavelength between 570nm and 610nm.
7. Incubate for 5 minutes at room temperature and read the absorbance at 595nm.
8. Plot a standard curve of absorbance vs. concentration.
9. A 2000ug/ml of the Standard Curve Reagent concentrate is also provided. This can be diluted with clean deionized water to obtain other concentrations.

B. Determination of the Protein Concentration of Unknowns:

10. If the sample is in a solubilized state proceed to Step 11. If the sample is a crude homogenate or slurry it should be diluted in the supplied solubilization buffer, and incubated at room temperature for 15minutes with frequent vortexing. Centrifuge the mixture (12,000xg for 2minutes) to clarify the sample, and use the supernatant for the protein concentration assay. Take care not to over dilute the sample.
11. **Note:** The unknown protein concentrations should be within the range defined by the standard curve. If not sure, a small amount of sample (e.g. 10uL) can be added to a tube with 300uL of Quanti-Protein Assay Reagent to roughly check the color of the mixture. If the color is too dark the sample should be diluted with the supplied solubilization buffer prior to Step 12.
12. Transfer 10uL of each sample into a well in a microtiter plate. Prepare samples in duplicate or triplicate.
13. To each well of unknown, add 300uL of **Quanti-Protein Assay Reagent**.
14. Carefully mix as in Step 5.
15. Incubate for 5 minutes at room temperature and read the absorbance of each at 595nm.
16. Extrapolate the protein concentration of unknowns from the Standard Curve.

Note: **Steps A & B should be performed simultaneously in a single microtiter plate.**

Distributed Exclusively By:

ITSI Biosciences
210 Industrial Park Road, Suite 100
Johnstown, PA 15904, USA
Attention: Product Manager
Phone: 1-814-262-7331 Fax: 1-814-262-7334
Website: www.itsibio.com Email: itsi@itsibio.com