ITSI BIOSCIENCES
FAST, ACCURATE, EASY- TO-USE

TOTAL PROTEIN ASSAY USING THE *ITSIPREP*™ ToPA KIT

IMPORTANT: K-0014-20 is a validated kit and procedure carefully developed and prepared to help scientists easily and accurately determine the total protein concentration using the ToPA total protein assay kit. The standard ToPA kit contains all the reagents and materials required for protein quantitation using a spectrophotometer. The easy-to-follow procedure and Ready-to-Use reagents makes protein quantitation easy and reproducible. ToPA reagents are also more tolerant to common laboratory buffers.

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

MATERIALS PROVIDED IN THIS KIT (Sufficient for 20 Assays):

Item	Size	Catalog #	Storage
Quanti - Protein Assay Reagent (Q-PAR)	1 x 30mL	Cat #: K-0014-20.1	4° C
Standard Curve Reagents (SCR)	7 x 750uL	Cat #: K-0014-20.2	Rm. T.
2000ug/mL Standard Curve Reagent (SCR)	1 x 750uL	Cat #: K-0014-20.3	Rm. T.
Solubilization Buffer	2 x 1.0mL	Cat #: K-0014-20.4	-20° C
Semi Micro Cuvettes	20 x 1.5mL	Cat #: K-0014-20.5	Rm. T.
Micro Centrifuge Tubes	20 x 1.5mL	Cat #: K-0014-20.6	Rm. T.
Procedure			

MATERIALS REQUIRED BUT NOT SUPPLIED:

1. Vortex mixer
2. Adjustable pipettes
3. Spectrophotometer capable of reading wavelengths between 570nm and 610nm

PROCEDURE:

Preparation of the Standard Curve:

1. Before beginning, bring the Quanti-Protein Assay Reagent up to room temperature.
2. Vortex the Standard Curve Reagent tubes well to ensure they are completely mixed.
3. To prepare a standard curve, pipette 20 µL of each **standard** into an appropriately labeled 1.5ml micro centrifuge tube. The standards should be made in duplicate at minimum.
4. Mix the Quanti-Protein Assay Reagent well by inverting the bottle (do not shake), and add 1mL of **Quanti-Protein Assay Reagent** to each standard.
5. Cap the tube and vortex briefly to mix.
6. Zero the spectrophotometer at 595nm with ~1mL of clean distilled water. If 595nm is not possible any wavelength between 570nm and 610nm may be used.
7. Read the absorbance of each mixture (standard + Assay Reagent) after 5 minutes of incubation at room temperature by transferring the mixture from the micro centrifuge tubes to a 1.5ml semi micro cuvette (provided)
8. Plot a standard curve of absorbance vs. concentration.
9. A 2000 µg/ml of the Standard Curve Reagent concentrate is also provided and can be diluted with deionized water to make other dilutions.

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 for 15min 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 that** the unknown protein concentrations need to be within the range defined by the standard curve. If not sure, a small amount of sample (around 10 µL) can be added to a tube with 300 µL of Quanti-Protein Assay Reagent to roughly check the color of the reagent. If the color is too dark the sample should be diluted with the supplied solubilization buffer prior to Step 12.
12. Once the sample is ready to assay, pipet 20 µL of each sample to an appropriately labeled 1.5ml micro centrifuge tube.
13. To each micro centrifuge tube add 1mL of **Quanti-Protein Assay Reagent**, cap and vortex the tube.
14. Incubate for 5 minutes at room temperature, then transfer the unknowns from the micro centrifuge tube to a 1.5ml semi micro cuvet (provided), and measure the absorbance at the same wavelength that was used for the standard curve.
15. Plot the reading of the unknowns against the standard curve to obtain the unknown protein concentration.

***Conditions for use of this procedure/Buffers:**
This VBP is the intellectual property of ITSI Biosciences. Only complete set of reagents provided by ITSI Biosciences should be used when possible because their compatibility with the downstream application has been validated. Considering that many factors can cause experiments to fail, ITSI Biosciences cannot guarantee that the use of this VBP and buffers will lead to a successful experiment. In no event shall ITSI Biosciences be held liable for loss of samples, failure of experiments or any other damage or injury associated with the use of this procedure or associated materials and reagents.

***General Safety Information and conditions for using the product:**
Consider all chemicals as potentially hazardous. Only trained laboratory personnel familiar with good laboratory practice should handle this product. Protective clothing should be worn. Use caution to avoid contact with skin and eyes. If contact should occur, wash immediately with plenty of water and follow established guidelines/procedures in your laboratory. **Warning: The procedure and kit are intended for research use only, not for use in human, therapeutic, or diagnostic applications. While ITSI will replace all defective products, it does not accept any responsibilities for improper use of this product, or loss/damages to samples. The end user is responsible for all local, state and federal regulations associated with the use and disposal of laboratory reagents.**

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