ITSI BIOSCIENCES

TOTAL PROTEIN ASSAY USING THE ToPA-nano KIT AND NanoDrop™* ND-1000 OR 8000 SPECTROPHOTOMETERS

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IMPORTANT: K-**0020-10** is a validated kit and procedure developed specifically for the NanoDrop™ ND-1000 and ND 8000 spectrophotometers. The NanoDrop™ is a full-spectrum UV-V is (220-750nm) spectrophotometer with a wide dynamic range. It does not require cuvettes or capillaries making it the most ideal spectrophotometer when sample is scarce. The ToPA-nano™ kit is prepared to help scientists easily and accurately determine total protein concentration using as little as 2uL of each sample. ToPA-nano™ is similar to the standard Bradford, but it is more sensitive and accurate than the Bradford reagent when used on the NanoDrop. This means that the "Protein Bradford" application module available in the NanoDrop™ can be conveniently used. The standard ToPA-nano™ kit contains all the reagents, including a standard blank reagent, room temperature stable ready-to-use protein standards and assay tubes required for total protein quantitation. ToPA-nano™ provides you the convenience of completing protein assays in less than 10 minutes. The easy-to-follow procedure and Ready-to-Use reagents makes protein quantitation easy, reproducible, faster and more cost effective. ToPA-nano™ 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 10 Assays):

Item	Size	Cat #	Storage
nQuanti-Protein Assay Reagent (nQ-PAR)	1 x 1.0mL	K-0020-10.1	4°C
Standard Blank Reagent (SBR)	1 x 1.0mL	K-0020-10.2	Rm. T.
Standard Curve Reagents (SCR)	8 x 250uL	K-0020-10.3	Rm. T.
Micro Centrifuge Tubes	30 x 0.5mL	K-0020-10.4	Rm. T.
Procedure			

MATERIALS REQUIRED BUT NOT SUPPLIED:

1. Calibrated adjustable pipettes and tips
2. NanoDrop™ Spectrophotometer ND-1000 or 8000.

- Before beginning, bring all reagents up to room temperature. This is necessary if they were stored at 4°C.
- Mix nQuanti-Protein Assay Reagent by inverting 2-3 times. **Do not shake vigorously or vortex. Do not dilute.**
- Adequately wipe the NanoDrop™ upper and lower sample measurement pedestals after each measurement.
- To ensure that no residual sample remains, clean the pedestals with 2uL of Standard Blank Reagent after each measurement.
- For accurate results the unknown protein concentrations need to be within the range defined by the standard curve.
- Only use Standard Blank Reagent for dilutions.

PROCEDURE:

Standard Curve Preparation:

1. Transfer 2uL of **Standard Blank Reagent** into an appropriately labeled 0.5mL tube.
2. Mix the Standard Curve Reagents by inverting the tube 2-3 times. **Do Not Vortex.**
3. Transfer 2uL of each **Standard Curve Reagent** into appropriately labeled 0.5mL tubes. The standards should be prepared in duplicate at the minimum.
4. Add 30uL of **nQuanti-Protein Assay Reagent** to all tubes. Mix by pipetting up and down 2-3 times.
5. Incubate at room temperature for 5 minutes.
6. Blank the instrument at 595nm by transferring 2uL of Standard Blank Reagent (tube "0") to the lower sample measurement pedestal of the NanoDrop™.
7. Next, read each reacted Standard Curve Reagent (Standard Curve Reagent + nQuanti-Protein Assay Reagent) by transferring 2uL directly unto the NanoDrop™ lower sample measurement pedestal. **Wipe the sample pedestal after each measurement.**
8. Plot a standard curve of absorbance vs. concentration.

NOTE: A 2000ug/mL of the Standard Curve Reagent concentrate is provided. This can be diluted with Standard Blank Reagent to make other concentrations of Standard Curve Reagents.

Determination of protein concentration of samples:

9. Pipette 2uL of each sample to an appropriately labeled 0.5mL tube provided.
10. Add 30uL of **nQuanti-Protein Assay Reagent**. Mix by pipetting up and down 2-3 times.
11. Incubate at room temperature for 5 minutes.
12. Read each mixture (nQuanti-Protein Assay Reagent + sample) by transferring 2uL directly unto the NanoDrop™ lower sample measurement pedestal.
13. Extrapolate the protein concentration from the standard curve (Step 8) to obtain the unknown protein concentration.

NOTE: The NanoDrop™ software can automatically generate the 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 when working in the laboratory. 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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