ISO 9001/14001 Certified Company

PRO-MEASURE™ Protein Measurement Solution

Cat. No. 21011 100 ml

DESCRIPTION

Protein quantification is one of the important steps in protein research such as Western blot assay. Protein assay methods are diverse, and there is one which absorbs at 280nm without special reagents or operation. This method is simple, yet it also has a shortcoming not being able to analyze protein assay when no amino acid such as phenylalanine, tryptophan or tyrosine are present. Neither will it be used commonly due to its DNA absorbance interference (AI). The other methods are Lowry assay or BCA assay, which are highly sensitive for protein quantification, but is quite inconvenient and time-consuming. Contrarily, Bradford method is the most commonly used one (within 10min), which can detect the minimum amount of protein and is very simple to use. This PRO-MEASURE™ is even more convenient than Bradford assay, declining AI of solution itself so that the background absorbance is maintained very low.

CHARACTERISTIC

- Very simple usage steps
- Short measurement time within 10min.
- High sensitivity to detect 1-250 μg/ml protein.
- Lower background interference.
- Able to to quantify protein by minimum amount of reagents.

CONTENTS

• PRO-MEASURE™ solution (10x)	100 ml
• Standard solution (BSA, 1 mg/ml)	1 ml

STORAGE

Store at 4°C, and then stable for more than 1 year.

PREPARATION BEFORE USE

Before use, dilute 1 part PRO-MEASURE™ solution by adding 9 parts of sterile water. For example, if you are to produce 50 ml dilution solution, mix 5 ml PRO-MEASURE™ with 45 ml sterile water.

CONSIDERATION BEFORE USE

1. In protein quantification, there can be an error due to high AI, depending on the solution type used for protein assay. Therefore, one must perform calibration using a blank (background absorbance) to nullify possible errors. For example, it has to be handed carefully if using a solution containing detergents such as Triton X-100, NP-40, deoxycholate etc., for they effect the absorbance of protein assay. iNtRON PRO-PREP™ (Cat. No.17081) can extract protein without affecting the protein assay.
2. In protein assay, one must use the original solution for dilution. To keep an error to minimum, one can perform dilution by using the protein extracting solution or using sterile water if necessary.
3. When measuring absorbance, use plastic cuvette. Because, this solution can coat the surface of glass cuvette. As the results, this solution give effect for absorbance measurement. PRO-MEASURE™ solution on glass cuvette surface can be removed by MtOH washing.
4. When quantification of protein, normally assay in duplicate or triplicates. Because we can decrease an measurement error.
5. For each protein measurement, one should calculate standard curve. By the way, the equation of iNtRON is suitable for any experiment with relative equal volume (Western blot, etc).
6. When measuring absorbance at 595nm, you can use cuvette or 96well-micro plate.
7. Suitable reaction time is within 2-10 min after the addition of samples. Absorbance will increase over time, solution should incubate at room temperature for no more than 1 hour.

PROTOCOL

Following method is brief summary.

1. Prepare sample solution of 100 μl through dilution of protein.
2. Add 1 ml of diluted PRO-MEASURE™ solution.
3. Incubate for 2 min at RT, measure absorbance at 595 nm.
4. Calculate concentration of protein by formula.

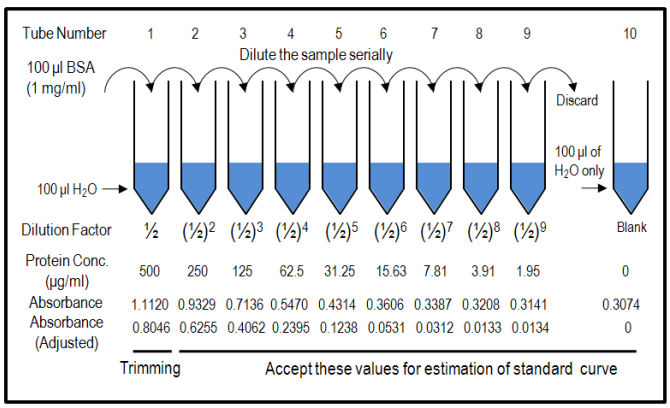
STANDARD CURVE

- This experiment method is to determine standard curve for BSA (bovine serum albumin) (Figure provided).
- The standard curve presented in this manual can be varied depending on environment and equipment of the actual experiments, and path-length of cuvette. Therefore we recommend creating standard curve every experiment.

1. Prepare two sets of 1.5 ml tubes each set with 10 tubes, add 100 μl of sterile water.
2. Add 100 μl of BSA solution to 1st tube and mix, 100 μl of solution transfer by pipetting from this tube(1st tube) to 2nd tube(1/2 dilution). Prepare for until number 9 tube by 1/2 dilution method. Number 10 tube add 100 μl of dH_2O, apply to as blank control.
3. Add 1 ml of PRO-MEASURE™ dilutes to each tubes, incubate for 2 min, measure absorbance at 595 nm.
4. Calculate standard curve through OD_{595} and concentration of BSA.
5. Introduce to equation ; Y (protein concentration) = 500 x A_{595} – 13.5

[Step 1]
Add 100 μl of dH_2O to each tubes and dilute serially by addition of 100 μl of BSA solution.

[Step 2]
Add 1 ml of diluted PRO-MEASURE™ solution, measure at 595 nm after incubating for 2 min.



[Step 3]
Calculate standard curve through OD_{595} and concentration of BSA.