SPN™-Protein Assay

INTRODUCTION

SPN™-Protein Assay is a fast and efficient spin column method for protein estimation. It requires only 0.5 – 10µg proteins per assay and no protein standards are needed. The assay is suitable for a wide range of protein samples including detergent solubilized membrane proteins and is compatible with common laboratory agents such as reducing sugars, thiols, chelating agents, and detergents including SDS up to 2%. The concentration of protein is determined by comparing the optical density (OD_{595}) data with the reference data on the supplied SPN™ Tables. The protein concentration can be determined in less than 10 minutes. The SPN™ Tables have been prepared using Bovine Serum Albumin (BSA) protein standards. For researcher's convenience, we have included protein standard ODs for both spectrophotometer and microplate reader. The kit components are enough for 50 protein assays.

ITEMS SUPPLIED	**Cat. # 786-020**
SPN™ Column	50
SPN™ Assay Dye	5 ml
SPN™ Wash Buffer-I	10 ml
SPN™ Wash Buffer-II	50 ml
SPN™ Elution Buffer	50 ml
SPN™ Tables I & II: Lot Specific (**with / without SDS**)	2 Tables

STORAGE CONDITION:

Shipped at ambient temperature. Upon arrival, store the kit components at room temperature. When stored and used properly, this kit is good for 12 months.

ITEMS NEEDED AND NOT SUPPLIED WITH THIS KIT:

Centrifuge, 2ml collection tubes and disposable polystyrene cuvettes (*G-Biosciences,* Cat # 786-009)

PROTOCOLS:

A. Single SPN™ column protein assay:

1. Put a SPN™ Column on a 2ml collection tube. Load 1-10µl protein samples (not to exceed 10µg protein) to the white solid matrix of the SPN™ Column.
2. Add 100µl SPN™ Wash Buffer-I to the SPN™ column. Centrifuge 5,000x g for 10 seconds to let the buffer pass through the matrix completely. Repeat the above wash once.
3. Add 100µl SPN™ Assay Dye to the SPN™ Column. Incubate 1-2 minutes at room temperature.
4. Centrifuge 5,000x g for 10 seconds to let the free SPN™ Assay Dye drain out of the column.
5. Change the collection tube. Add 500µl SPN™ Wash Buffer-II to the column. Centrifuge 5,000x g for 10 seconds to let the buffer pass through the matrix completely. Repeat the above wash once.
6. Put the SPN™ Column in a new collection tube. Add 250µl SPN™ Elution Buffer to the column. Centrifuge 5,000x g for 10 seconds to let the buffer pass through the matrix completely. Repeat the elution once and collect the elution in the same collection tube.

NOTE: *For measuring absorbance using microplate reader, see section B. For measuring the absorbance using spectrophotometer, continue with step 7.*

7. Discard the SPN™ Column. Add 500µl SPN™ Elution Buffer to the collection tube and mix. Read the absorbance at 595nm against deionized water using 1cm optical path length cuvette.
8. Use the SPN™ Table for Spectrophotometer to determine the amount of protein in your sample. Calculate the protein concentration (µg/µl) by division of the amount of protein (µg) read from the table by the protein sample volume (µl) loaded to the spin column.

NOTES:

I. The OD_{595} values provided on SPN™ Tables I & II for Spectrophotometer were measured using 1cm optical path length cuvette, and deionized water as blank for fast and convenient determination. You need to make your own standard plot if you use different optical path length cuvette.

II. If your protein sample contains SDS, use SPN™ Table-II Samples containing up to 2% SDS.

III. Setting up duplicate columns for each assay will improve the accuracy of your protein estimation.

B. Using microplate reader to determine the protein concentration:

1. Mix the eluent from SPN™ Column (step 6 of section A) and transfer 200µl of it to one well of 96-well plate.
2. Read the absorbance at 595nm with a microplate reader, using deionized water as blank.
3. Use the SPN™ Table For Microplate Reader to determine the amount of protein in your sample. Calculate the protein concentration (µg/µl) by dividing the amount of protein (µg) read from the table by the protein sample volume (µl) loaded to the spin column.

NOTES:

I. The absorbance values on SPN™ Tables I & II For Microplate Reader supplied with the kit were measured using Nunc-Immuno™ Plate, MaxiSorp™ Surface, Cat# 442404, Nalge Nunc International, 80045LE0702, and deionized water was used as blank for fast and convenient determination. The absorbance values may vary if a different type of 96-well plate is used and in that case you need to make your own protein standard plot.

II. If your protein sample contains SDS, use SPN™ Table-II Samples containing up to 2% SDS.

III. Setting up duplicate columns for each assay will improve the accuracy of your protein estimation.

RELATED PRODUCTS

1. Non-Interfering Protein Assay™ (Cat.# 786-005): *NI-Protein Assay™ is not affected by interfering agents commonly present in protein solutions, including reducing agents such as 2ME, DTT, detergents, amines, EDTA, Salts, sugars etc. NI-Protein Assay™ also shows no protein-to-protein variation. The assay is based on removal of interfering agents prior to assay by a single step protocol. The sensitivity is as low as 0.5ug/assay. Assay time is 15-20 minutes*

2. dotMETRIC™ - 1µl Protein Assay (Cat.# 786-20/21): *For sample economy and rapid estimation of protein using a test strip. Simply apply 1µl protein solution on the test strip, develop test strips in 8 minutes, and measure the diameter of protein spot on test strip for determination of protein concentration. dotMETRIC™ assay is resistant to reducing agents, detergents and shows little or no protein to protein variation.*

3. Ready-to-Screen Tissue Blots for Western Analysis: *G-Biosciences has put together pre-made Ready-to-Screen Tissue Blots of total proteins extracted from hard-to-obtain tissues from human (normal, tumor and region specific), mouse, rats and cell lines, etc. (For details please visit our web site at www.GBiosciences.com)*

4. Western Re-Probe™ (Cat # 786-119): *Western Re-Probe (5X) kit provides buffer for stripping and re-probing Western blot membranes.*

5. NAP-Blocker™ (Cat # 786-190): *It is a blocking agent (2X) containing non-animal proteins for improved assay sensitivity, clear background and a high signal to background ratio. Better results than milk powder preparation as it ensures uniform blocking without non-specific binding.*

6. Bovine Gamma Globulin Standard (2mg/ml) (Cat # 786-007): *Protein standard for protein estimation.*

Note: For other related products, visit our web site www.GBiosciences.com or contact us.