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A Geno Technology, Inc. (USA) brand name

SPN™ Protein Assay

(Cat. # 786-020)

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 ≤2% SDS.

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 <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.

ITEM(S) SUPPLIED (Cat. # 786-020)

Description	Size
SPN™ Columns	50
Mini Collection Tubes	50
SPN™ Assay Dye	5ml
SPN™ Wash Buffer-I	10ml
SPN™ Wash Buffer-II	50ml
SPN™ Elution Buffer	50ml
SPN™ Tables I & II: Lot Specific (with/without SDS)	2 Tables

STORAGE CONDITIONS

The kit is 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.

ADDITIONAL ITEMS REQUIRED

Centrifuge and disposable polystyrene cuvettes (*G-Biosciences,* Cat. # 786-009)

PROTOCOL

A. Single SPN™ column protein assay

1. Place a SPN™ Column in a 2ml Mini 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 5,000xg 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 5,000xg 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 5,000xg 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 5,000xg 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, SD ±5%. 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.
   NOTE: *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.*
   NOTE: *If your protein sample contains SDS, use SPN™ Table-II Samples containing up to 2% SDS.*
   NOTE: *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, SD ±5%. 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.

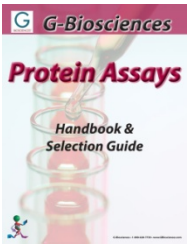
   NOTE: *The absorbance values on SPN™ Tables I & II For Microplate Reader supplied with the kit were measured using Nunc-Immuno™ Plate, MaxiSorp™ Surface, Cat# 442404, Nalge Nunc International, 80045LE0702, and deionized water was used as blank for fast and convenient determination. The absorbance values 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.*

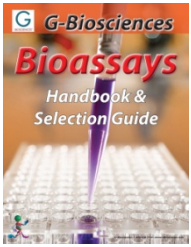
   NOTE: *If your protein sample contains SDS, use SPN™ Table-II Samples containing up to 2% SDS.*

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

RELATED PRODUCTS

Download our Protein Assays or Bioassays Handbooks.





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