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A Geno Technology, Inc. (USA) brand name

SPN™-htp Protein Assay

(Cat. #786-021, 786-900)

INTRODUCTION

SPN™-htp Protein Assay is a fast and efficient spin plate method for protein estimation. It requires only 0.5 - 10µg protein per assay. 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. The kit components are enough for 480 protein assays.

ITEM(S) SUPPLIED (Cat. # 786-021)

Description	Cat. # 786-021	Cat. # 786-900
SPN™-*htp* Plate 96 well	5	5
SPN™ Collection Plate 96 well	6	6
SPN™ Assay Dye	50ml	50ml
SPN™ Wash Buffer-I	100ml	100ml
SPN™ Wash Buffer-II	2 x 250ml	2 x 250ml
SPN™ Elution Buffer	2 x 125ml	2 x 125ml
Bovine Serum Albumin (BSA) Standard [2mg/ml]	5ml	-
Non-Animal Protein Standard [2mg/ml]	-	5ml

STORAGE CONDITIONS

The kit is shipped at ambient temperature. Store Protein Standard at 4°C and store other kit components at room temperature after receiving. When stored and used properly this kit is good for 12 months.

ITEMS NEEDED AND NOT SUPPLIED WITH THIS KIT:

Centrifuge, 96-well plate and plate reader

PREPARING DILUTED PROTEIN STANDARD

It is recommended to prepare the appropriate diluted protein standard (0.1-1.0µg protein/µl) in the same buffer diluent used for the test protein samples.

Stock 2mg/ml Protein Standard	Diluent	Final Protein Concentration
Volume added in µl	**Volume added in µl**	**µg/µl**
0	200	0
10	190	0.1
20	180	0.2
30	170	0.3
50	150	0.5
80	120	0.8
100	100	1.0

PROTOCOL

1. Perform assay with duplicate protein standards and samples as follows:
2. Put a SPN™-*htp* Plate on a SPN™ Collection Plate (Can be reused for waste collection). Load 10µl diluted protein standard to the white solid matrix of the SPN™-*htp* Plate.
3. Load 1-10µl protein samples (not to exceed 10µg protein) to the white solid matrix of the SPN™-*htp* Plate.
4. Add 200µl SPN™ Wash Buffer-I to each well of the SPN™-*htp* Plate. Centrifuge 2,000 x g for 30 seconds to let the buffer pass through the matrix completely.
5. Add 100µl SPN™ Assay Dye to each well of the SPN™-*htp* Plate. Incubate 2 minutes at room temperature.
6. Centrifuge 2,000x g for 30 seconds to let the free SPN™ Assay Dye drain out of the plate.
7. Add 500µl SPN™ Wash Buffer-II to each well of the SPN™-*htp* Plate. Centrifuge 2,000x g for 30 seconds to let the buffer pass through the matrix completely.
8. Pour off the waste in the SPN™ Collection Plate and put it back under the SPN™-*htp* Plate. Repeat the above step 6 wash once.
9. Put the SPN™-*htp* Plate on a new SPN™ Collection Plate. Add 500µl SPN™ Elution Buffer to each well of SPN™-*htp* Plate. Centrifuge 2,000x g for 30 seconds to let the buffer pass through the matrix completely.
10. Mix the eluent in the SPN™ Collection Plate by pipetting up and down 3-4 times. Transfer 200µl of it to one well of 96-well plate.
11. Read the absorbance at 595nm with a microplate reader.

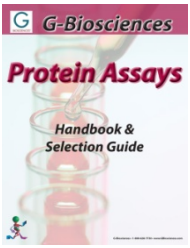
12. Plot a standard curve for determination of protein concentration of unknown samples.
13. ***Note:*** *Microsoft Excel can be used for determination of the protein concentration.*
14. *Draw standard XY scatter curve with protein amount (µg) as x values and absorbances as y values. Add trendline. Select polynomial type with order of 2 and display the equation on the chart. Use the equation to determine the amount of protein (µg) in each assay and calculate the protein concentration (µg/µl) by dividing the amount of protein (µg) by the volume loaded to the SPN™-htp Plate (µl).*

B. SONICATION & GLASS BEADS ASSISTED EXTRACTION

15. Suspend clean yeast cell pellet in 5-10 times volume of prepared FPS Buffer. Add 5 times volume of glass beads (0.5 mm diameter). Sonicate the suspension with an ultrasonic probe to break the cells and break down the genomic DNA. Sonication should be performed in cold (ice cold bath) and during sonication care must be taken to prevent heating. Sonication should be performed with bursts of 30-40 seconds and chill the suspension between ultra-sonic bursts. It may take 10 - 30 minutes depending on the sample size and the sonication strength. Other mechanical devices and grinders may be used instead of ultrasonic probe.
16. Centrifuge the homogenate at 20,000x g for 30 minutes at 20°C to pellet the debris and collect the clear lysate.
    Optional: *Suspend the residual cell debris in 1/4 the volume of FPS Buffer used in the previous Step-1. Sonicate the suspension once briefly. Repeat Step 2. Collect the extract and pool with the first extract supernatant. Store total protein extract at -70°C until used.*
17. Determine protein concentration. We recommend Non-Interfering Protein Assay, (Cat. # 786-005).
18. Make an appropriate dilution in FPS Buffer before running IEF/2D gels.
    Note: *Proteins solubilized in FPS Buffer that contain CHAPS may not suitable for running SDS-page electrophoresis. Use PAGE-Perfect (G-Biosciences Cat #786-123) to remove detergent before running SDS-page electrophoresis.*

RELATED PRODUCTS

Download our Protein Assays Handbook.



http://info.gbiosciences.com/complete-protein-assay-guide?hsCtaTracking=d958be55-c2e5-4e08-9616-7c3d82e38c57|ba96d0df-fe94-4a7a-af86-f2798cf4f55d

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