Dot·it·
Spot·it

Dot-it-Spot-it Total Protein Assay

Instructions issued October 2017

SUMMARY AND EXPLANATION

Sample is applied on the Detection Sheet, which has 24 positions for 1 µL aliquot. The sheet is dried and the proteins in the sample are bound to the sheet. A Detection Solution containing carbon black particles is flowed over the sheet, and binds to the proteins. After washing and drying the sheet is mounted on a template and the blackness intensity in the 24 dots is detected by the use of an image scanner.

REQUIRED ITEMS

Included in the Dot-it-Spot-it kit	Detection Sheet Detection Solution Washing Solution Mounting Template
Equipment included in the Start-up package	Light source, reservoirs Dispensing template, clips, tape roller
Other equipment	Pipettes, hair dryer
Detection equipment	Image Scanner *(Epson Perfection 600 Photo - or similar)*
Software	ImageJ is recommended
To download: www.dot-it-spot-it.com/method/	- Template for calculation - Detection recommendation

Technical Assistance

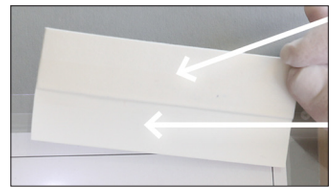
If you have any problems or experience any difficulties regarding the products, please do not hesitate to contact us.

Web: www.dot-it-spot-it.com
Email: info@dot-it-spot-it.com
Mail: Maplestone AB, Högåsvägen 205, SE-74141 Knivsta, Sweden

PROCEDURE

Precautions Touch only the thicker pad mounted on the Detection Sheet. Be aware of that the thinner nitrocellulose sheet will directly capture proteins from your hands, from traces on your gloves, and from dust.

Even tiny drops of saliva can be seen on the sheet after a good laugh - it is really a protein sensitive technique!



Detection Sheet:
Thick pad: You can touch and write on it.

The thin nitrocellulose sheet: Be careful, do not touch – it adsorbs proteins.

1. Sample mixture

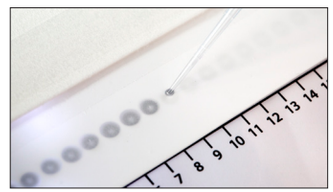
Mix your sample and standard set with the Dilution buffer which is provided together with the kit. Standard set: make a dilution series of e.g. BSA using your sample buffer.
- Low protein range (50% sample): use 0.4-10 µg BSA/mL as standard and mix 2 µL sample/standard with 2 µL Dilution buffer.
- Medium protein range (10% sample): use 2-50 µg BSA/mL as standard and mix 1 µL sample/standard with 9 µL Dilution buffer.
- For higher concentrations: dilute the sample and standard in your sample buffer to the medium protein range, and mix 1 µL sample/standard with 9 µL Dilution buffer.

2. Position the Detection Sheet on the dispensing template

Position the Detection Sheet on the Dispensing Template and place a light source under. Then you can see the 24 circles on the template through the thin nitrocellulose part of the sheet.

3. Dispense sample mixture and dry

Dispense 1.0 µL of sample mixture on the nitrocellulose sheet in the designed circle shown by the template. Dry the wet spots carefully with a hair dryer in about 1 min, don't use temperature above 75°C.



3. Detect and wash

Place the nitrocellulose end of the sheet in a reservoir with 1 mL of Detection Solution.

After 5 min. move the sheet to a reservoir with 1 mL of Washing Solution. After 5 min. take up the sheet.

4. Dry and mount on Mounting Template

Take up the Detection Sheet, remove the thicker pad, and let the nitrocellulose sheet dry, remove tape, and finally mount it on the Mounting Template using the tape roller.

5. Scan

Place the Mounting Template with the Detection Sheets in the recommended position for A4 on the scanner.
Save the scan as a TIFF image.
For details see "Detection recommendation" on www.dot-it-spot-it.com/method/.

6. Concentration estimation

Calculate the concentration for the unknown samples by comparing signal values (dbpp) with values for the calibration samples.
For details see "Detection recommendation" on www.dot-it-spot-it.com/method/.