

Dot·it· Spot·it

Dot-it-Spot-it Total Protein Assay

Instructions issued March 2019

SUMMARY AND EXPLANATION

Sample is applied on the Detection Sheet, which has 18 positions for 1 μ L 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 18 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 Dilution Buffer
Equipment included in the Start-up package	Light source, reservoirs Dispensing template, clips, tape roller
Other equipment	Pipettes
Detection equipment	Image Scanner (<i>Epson Perfection 600 Photo - or similar</i>)
Software	ImageJ is recommended
To download: www.dot-it-spot-it.com/method/	- Template for calculation - Detection recommendation

WARRANTY

Information presented here is accurate to the best of our knowledge. It is the responsibility of the user to verify the suitability of the supplied materials and procedures for a particular purpose. In this respect further processing made by the user may affect the results, in which event Maplestone AB disclaims all warranties expressed, implied or statutory, including the implied warranty of merchantability and fitness for use. Maplestone AB and its authorised distributors, in such event, shall not be liable for damages indirect or consequential. The product is intended for research only.

Technical Assistance

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

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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. Application 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 0.2-10 μ g BSA/mL as standard and mix 2 μ L sample/standard with 2 μ L Dilution Buffer.

- Medium protein range (10% sample): use 1-50 μ g BSA/mL as standard and mix 1 μ L sample/standard with 9 μ L Dilution Buffer.

- For higher concentrations: dilute the sample and standard in your sample buffer to the medium protein range, and mix 1 μ L sample/standard with 9 μ L 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 18 circles on the template through the thin nitrocellulose part of the sheet.



3. Dispense sample mixture and dry

Dispense 1 μ L of application mixture on the nitrocellulose sheet in the designed circle shown by the template. Let the sheet dry for 30 min in room temperature. Thereafter use a brush to gently remove the dust.



3. Detect and wash

Place the nitrocellulose end of the sheet in a reservoir with 1 mL of Detection Solution. Wipe off the surplus of liquid at the end of the sheet.

After 5 min. move the sheet to a reservoir with 1 mL of Washing Solution. After 5 min. take up the sheet. Wipe off the surplus of liquid at the end of the sheet.

4. Dry and mount on Mounting Template

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/.