Dot·it· Spot·it

Dot-it-Spot-it Total Protein Assay

Detection recommendations, issued: June 2015

Easy-to-use detection instruction

Recommended scanner   Epson Perfection V600 Photo[1].
Epson Scan with no colour correction

1. Make sure that both scanner area and Detection sheets are dust free. Place the Detection sheets against the glass surface, on the area intended for A4 sheets.
2. Ensure correct settings in used scanner software. When using the scanner for quantification purposes it is important to obtain an image that is not auto corrected. Bit depth: 16-bit grey scale. Pixel resolution: 600 dpi.
3. Does your scanner need warm-up? Scanners with LED lamp can be used directly.
4. Scan each Detection Sheet and save the TIFF-files for further processing.

SCANNER

A good warmed-up scanner shows a low variation in the quality-controlled area with a CV of less than 1-2% when repeatedly measuring delta blackness per pixel on the strips.

Scanner Quality

The imprecision of the scanner measurement depends on the quality of the light source and CCD-elements. The variability in obtained signal intensity, delta blackness per pixel), has to be checked. Generally, the outmost peripheral parts of the scanner area may show aberrant values.

Resolution

Pixel resolution, 600 dpi

A resolution of 600 dpi (1 pixel = 0.042 mm) gives a sufficient number of measurement points.

Bit depth, 16-bit

A 16-bit grey scale bit depth (equal to colour 48-bit) gives blackness intensity values between 0 and 65,535 (2^{16}-1), performing a wide measuring range for the protein assay.

Warm-up

In addition to the initial start-up self-test the scanner, especially the lamp needs a certain warm-up period before accurate measurement can be done.

Make a scan and wait 5 minutes for the scanner lamp to be properly warmed up. Perform an additional scan before beginning of measurements.

Scanners with Ready scan LED technology from Epson does not need warm-up.

Detection Sheet Position

Make sure the sheet is aligned with the document table on your scanner, preferably using the edges of the document table glass, and located inside the area intended for A4 / LTR sheets.

Control of Scanner Imprecision

The scanner imprecision can be checked by scanning mounted sheets over and over again. Take out and re-position the sheet between each scan. Calculate the %CV for each spot. The selected scanning area should have a CV less than 1-2% at grey scale levels visible for the eye.

Cleaning

It is highly recommended to use the scanner solely as detection equipment and handle it as such to avoid interference problems from a dirty scanner surface.

Cleaning procedure

For smudges on the scanner glass surface use window cleaner on a soft piece of cloth or soft paper, as per scanner manual. For dust, and alike, use a lens cleaner brush or blower on the glass. The same procedure is recommended for the sheets as long as the brush is soft enough to not scratch or otherwise damage the porous layer on the strip.

SCANNER SOFTWARE

Scanner software is constructed to make possible easy generation of nice images and thus contains automatic functions for setting e.g. gamma value, histogram cropping brightness, contrast and sharpness. For quantitative detection such automatic settings should be avoided.

Gamma gradation

This is modification of the mid tone blackness in an image, aiming to increase the visibility for the human eye. A value of 1.0 gives an image without correction. Values of 1.8 and 2.2 are commonly used to give an image that shows more grey scale levels instead of nearly black tones.

Epson Scan (v. 3.9)

Works only with gamma gradation 1.8 or 2.2.

Settings

Mode: Professional mode

Document type: Photo

Image type: 16 bit gray scale

Resolution: 600 dpi

Adjustments: none

Configuration: Colour: No colour correction! Gives 1.8 gamma gradation.

Save

Image format: TIFF

The software can be downloaded free from Epson [1].

SilverFast (v. 8.2).

Can give a raw non-modified image when using gamma gradation 1.0.

Settings

Preferences: Gamma Gradation 1.00 ☐ for HDR output   (**not** ticked)

Scan Mode: Reflective

Image Type: Positive

Colour Depth: 16 Bit HDR Gray scale.

Scan dimensions

Output image in TIFF-format

Preset: 600 dpi

The software can be bought from LaserSoft Imaging [2]

CAMERA

Smart phone cameras are handy to use, but are, so far, available with only 8-bit values (256 grey scale). Iphone 5s has a 8 Mpixel camera, with a pixel size of 1.5 um. The gamma gradation seems to be around 1.8. If not used with a light box the light source may vary considerable. But used for only 1-2 strips with the sample and standard dots placed near to each other, it works quit good for quantification. Select the best light source, perhaps in daylight next to a window, and make a shot. Open the image in ImageJ, and follow the instructions found below.

HANDLING OF RAW VALUES

Blackness intensity measurement - ImageJ

Download the free software from http://rsbweb.nih.gov/ij/.

Shown below is one way to analyse the intensity in the spot.

Measure the reflected light intensity by:

1. Select a suitable size of the "circle" tool
2. Place the circle around the spot
3. Use the Analyze: Measure function
4. Place the circle below the spot for measuring the background.
5. A table is formed with the mean intensity of the reflected light
6. Copy the values in the table and paste into the "Dot-it-spot-it calculations" .xltx file, provided at www.dot-it-spot-it.com, that calculates delta blackness intensity.

Concentration determination

For determination of concentration in unknown samples additional statistic software is needed for suitable curve fitting of the protein assay calibration curve (e.g. four parameter logistic curve-fit or similar). Use the delta blackness per pixel values obtained from "Dot-it-spot-it calculations" file.

Example of useful statistic software:

- Image J, http://rsbweb.nih.gov/ij/
- Workout 2.0, Dazdaq Ltd, www.dazdaq.com
- R, R Development Core Team, www.R-project.org
- JMP 7, SAS Institute Inc, www.jmp.com

Concentration determination - ImageJ

http://rsbweb.nih.gov/ij/

ImageJ – Analyze – Tools – Curve fitting

Curve fitter:

1. Choose "Rodbard" curve fitting which is a four parameter general curve fit function proposed by David Rodbard at NIH. The form of the equation is: $y = d + (a - d) / (1 + (x/c)^b)$
2. Fill in Mean concentration and Mean signal intensity for each calibration points
3. Fit
4. Copy the obtained equation parameters (a-d) into the "Dot-it-spot-it calculation" file for concentration calculation of the samples.

RESULTS

Ocular detection

The eye sees very well differences in the blackness intensity. It easily identifies 2-times dilution; see Fig. 1, when having a reference curve on the same sheet. The set up, when using only 1-2 strips, takes about 15 minutes to perform, and is useful at several steps during lab work:

- To rapidly test and pick out the chromatography fraction that contains protein.
- To follow extraction procedures by frequently taking out sample for analysis.
- To verify that the sample you apply on the gel electrophoresis will be detectable with Coomassie Blue or silver staining.

Fig.1. Ocular detection. The Dot-it-Spot-it Detection sheet shows a dilution curve of human albumin, from 5 µg/mL down to 0.16 µg / mL, as noted below the triplicates. The eye can easily distinguish the different concentrations. This Detection sheet has also been used for the quantification with camera and scanner.

Smart phone camera

The camera captures and verifies what you see with your eye. The image from the camera can be used to calculate the concentration of unknown samples when you have reference controls included in the detection sheet. In Fig. 2 is seen the quantitative results for an image from IPhone 5s. The image of the Detection sheet was taken in daylight and the values are the mean from 8 shots, moving the camera in different positions, even upside-down. The variation in signal between the shots was low; mean CV was 3.7% in the signal range for 0.6-5 µg/mL.

This application is useful when having only a few samples, and is combined with software for quantification.
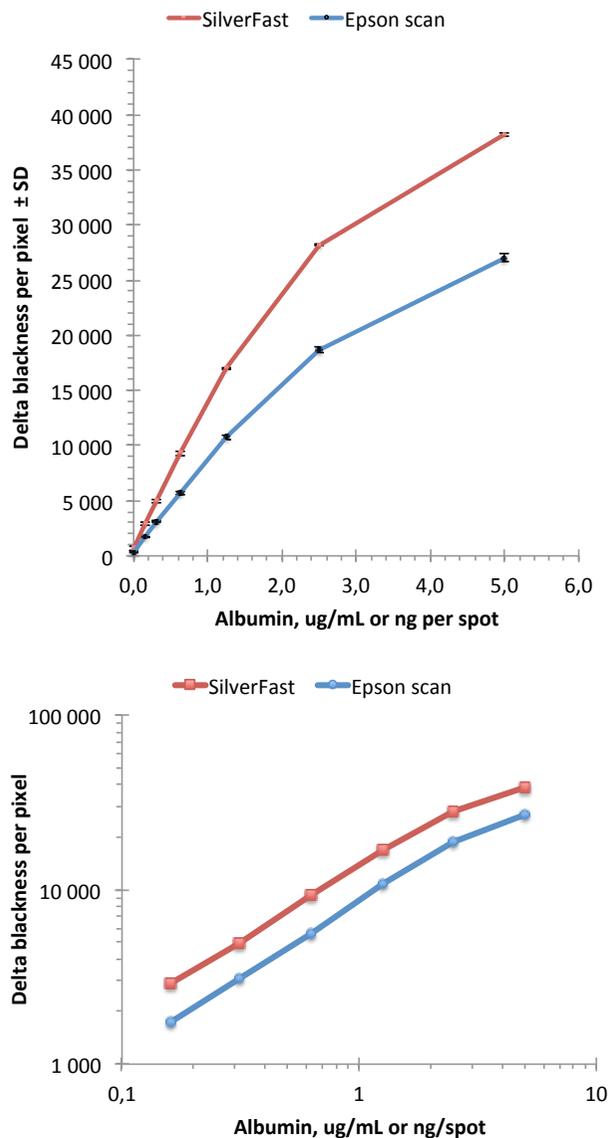
Scanners

Flatbed scanners are frequently found in laboratories for detection of stained proteins in electrophoresis gels, or for proteins blotted onto membrane sheets. Primarily the migration position has been

detected, but also the stain intensity has been quantified. The advent of lateral flow immunoassay, especially with the black carbon black particles as an antibody label, proved that scanners fulfilled the roll as a good and sensitive quantitative detection tools (Lönnberg et al, Quantitative detection in the attomole range for immunochromatographic tests by means of a flatbed scanner. Analytical Biochemistry 293, 224-231, 2001).

For the detection of the dots in Dot-it-Sot-it Total Protein assay spots we have started in 2015 to use the Epson Perfection V600 Photo scanner. This CCD scanner has shown good homogeneity and reproducibility, and the LED light source makes it rapidly prepared for scanning. The price is quite affordable, about 230 euro. The scanner has been used together with Epson Scan, but the colour correction function has been turned off. In Fig. 3 is shown scanning of the Detection sheet in Fig.1, using 16-bit grey scale and 600 dpi resolution. The variation between the 8 scanning runs in different position was very low; mean CV was 1.8% in the signal range for 0.6-5 µg/mL.
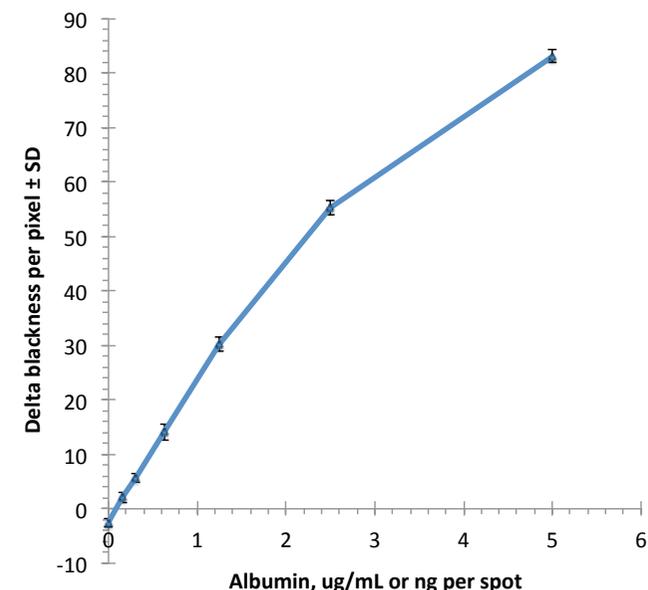
Fig. 2. Detection with IPhone 5s. The detection sheet shown in Fig. 1 was quantified by taking photos with the sheet placed in the daylight from a window. The variation in the signal between the shots was low, and the camera can be used for quantification, although best suited when using 1-2 Detection sheets.

Fig. 3. Detection with Epson Perfection V600 Photo.

The variation between scanning positions is very low for a scanner as shown by the almost invisible SD variation on the y-scale. Two softwares for scanning is compared in the plots, illustrated in lin-lin and log-log scale. The raw values from SilverFast, using a gamma value of 1.0, are compared with Epson scans gamma 1.8 corrected values, which gives lower signal intensity. Both types of software can be used as the calculation of unknown sample concentration is done by comparing the values with a standard curve.

D E T E C T I O N   T O O L S