DR6000 for Quantitative Determination of Proteins

By Ralf Kloos, Global Product Application Manager

Introduction:

Quantitative photometric measurements of proteins
There are a number of known photometric methods for quantitative determination of proteins that differ in terms of application, the measuring range and possible faults.

The following quantitative measurements of proteins are possible on the DR6000:

- Biuret
- Lowry
- Bicinchoninic acid
- Bradford
- UV280

Methods

Quantitative photometric measurements of proteins
The quantitative determination of proteins using photometry is often done on the basis of colour reactions of the functional groups of proteins with the corresponding dye reagents.

As with the photometric test for individual parameters, the intensity of the dye formed correlates directly with the concentration of the protein mixture and can be evaluated photometrically.
The samples are always measured against a blank value (mixture of reagents with distilled water instead of the protein sample on the same wavelength). A standard range based on a suitable standard (e.g. from bovine serum albumin) is used to calibrate the method and is measured alongside the protein samples.

If the standard curve is known from previous measurements, the known calibration factors can be entered instead of measuring a standard series again.

Biuret assay
The determination of the proteins based on the biuret assay describes the colour reaction of the protein mixture with dissolved biuret (carbamoylurea) in an alkaline, aqueous environment. The resulting red violet dye is measured in the photometer on a wavelength of 540 nm.

The biuret assay is considerably less sensitive compared with the following colour assay. Ammonium sulphate, glucose and sodium phosphate, for example, interfere with the assay.

The spectral absorption coefficient (mL final volume/µg dissolved protein) using 1-cm cuvettes is approximately $2.3*10^{-4}$ $A_{550\ nm}$.

The measuring range is approximately 10 µg/mL to 1200 µg/mL

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Lowry assay

Lowry and colleagues published a combination of the biuret reaction described above with a second reaction step (Folin-Ciocalteu phenol reagent; Lowry assay) for the first time in 1951. This considerably increased the sensitivity of the photometric detection of protein solutions. The deep blue colour that finally emerges is ideally measured at 750 nm.

However, the Lowry assay is susceptible to interference from a large number of detergents (EDTA, Triton X-100, SDS; Brij 35) and other additives to purify the enzymes (e.g. ammonium sulphate).
The different proportions of tyrosine, tryptophan, cysteine, cystine and histidine also contribute to different results.

The spectral absorption coefficient (mL final volume/µg dissolved protein) using 1-cm cuvettes is approximately $1.7*10^{-2}$ $A_{750\ nm}$.

The measuring range is approximately 1 µg/mL to 60 µg/mL.

Bicinchoninic acid assay

Smith and colleagues published an alternative to the Lowry approach in 1985, whereby the described biuret reaction was combined with bicinchoninic acid the as assay reagent. This approach is simpler to perform than the Lowry assay described above. The colour complex formed is measured on the photometer at 562 nm.

The bicinchoninic acid assay is also subject to a large number of interfering factors. In addition to EDTA and ammonium sulphate (> 5 %), other interfering factors include sucrose or glucose (> 10 mM), glycine, sodium acetate (2 mM) and sodium phosphate (1 mM).

The spectral absorption coefficient (mL final volume/µg dissolved protein) using 1-cm cuvettes is approximately $1.5*10^{-2}$ $A_{562\ nm}$.

The measuring range is approximately 1 µg/mL to 60 µg/mL.

Bradford assay

In this assay named after M.M. Bradford and published in 1976, blue acid dyes (Coomassie Brilliant Blue G250) react with the protein mixture. In an acidic environment, the maximum absorption of the dye shifts from 465 nm on a wavelength of 595 nm.

The Bradford assay is susceptible to interference from detergents such as Triton X-100 (> 0.5 %) and SDS (> 0.1 %).

The spectral absorption coefficient (mL final volume/µg dissolved protein) using 1-cm cuvettes is approximately $4.0*10^{-2}$ $A_{595\ nm}$.

The measuring range is approximately 1 µg/mL to 25 µg/mL.

Spectral absorption measurement at 280 nm (UV280)

Proteins have a maximum absorption at 280 nm, which is largely determined by the aromatic amino acids tryptophan, tyrosine and phenylalanine.

The UV measurement at 280 nm (in a quartz cuvette) is suitable only for significantly higher concentrations than the colour reactions previously described. As the contents of aromatic amino acids–

particularly tryptophan, which plays the main role in absorption–vary from protein to protein, the result is dependent on the protein used for calibration.
Again, a measurement must be taken against the corresponding blank value here, and calibration with the *pure* protein to be determined in the sample would be ideal.

The measuring range applied extends from approximately 50 µg/mL to 3000 µg/mL.

Measurements on DR6000:

The DR6000 handles the quantitative determination of proteins based on the corresponding method in the user program.
A standard series with appropriate standard proteins (BSA) must be created for the relevant method for the quantitative determination. In single wavelength mode, the appropriate wavelength and then the "Measure standards" function must be selected.

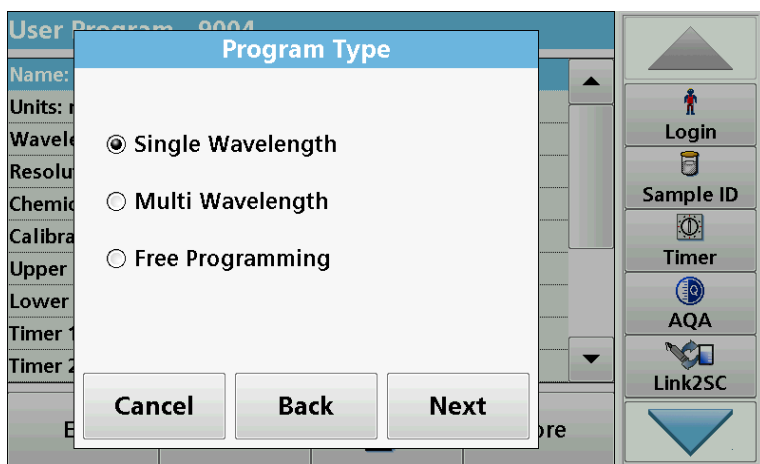The example below shows the Bradford method as user program 9004 by way of example.
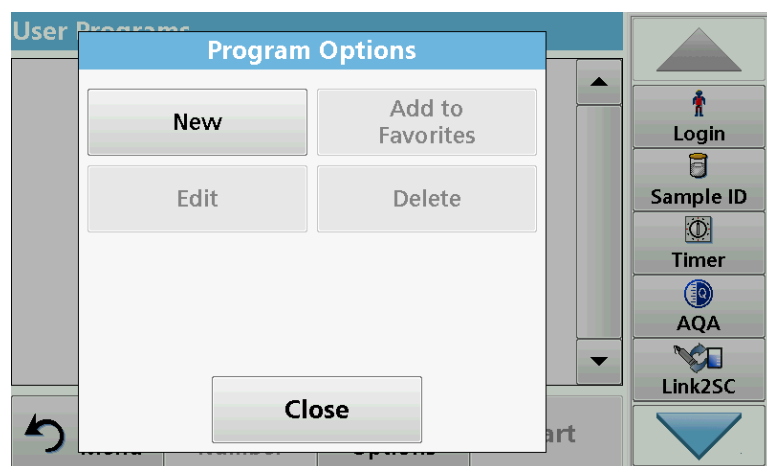
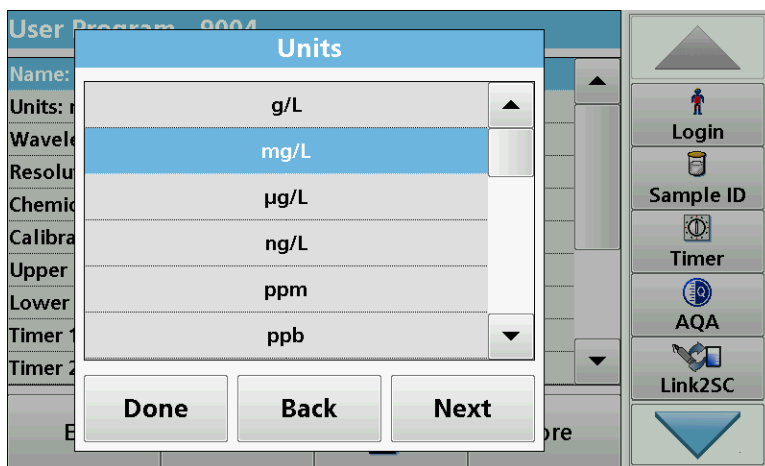Select **User Programs** → **Program Options** and **New**
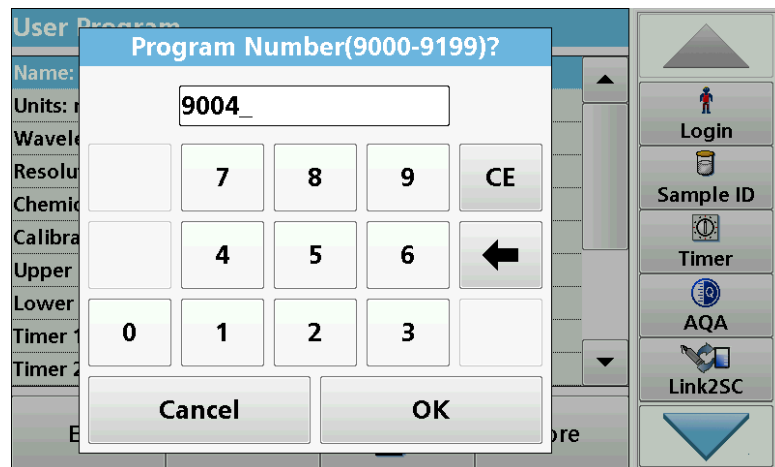
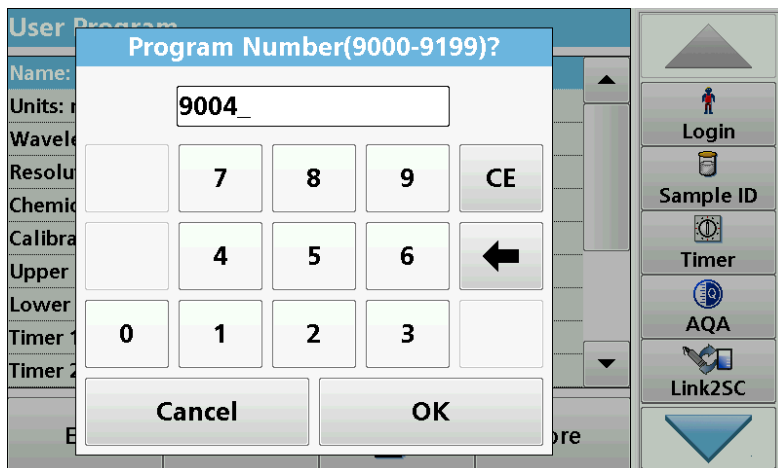Select a free program number.
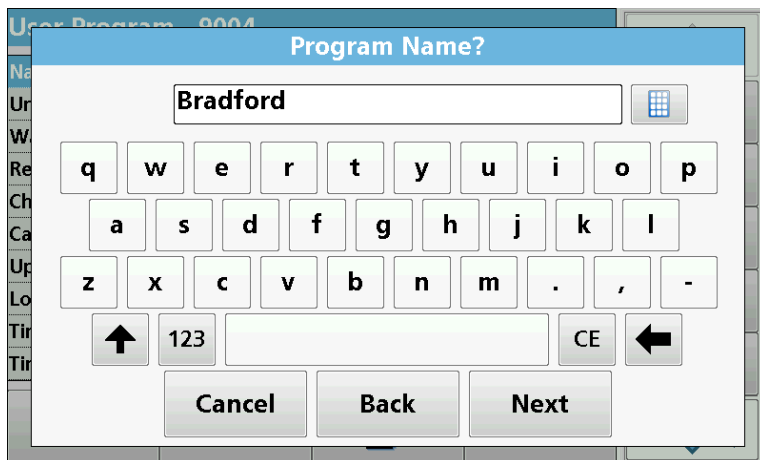
Select a suitable text name.

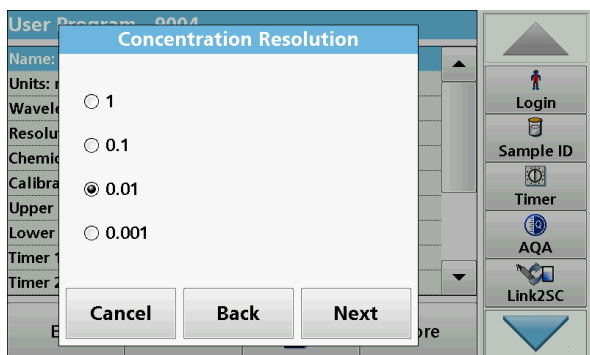Select the **Single Wavelength** programming mode.

Select **Units of Measure**.
Note: mg/L corresponds to the usual unit of measure of μg/mL
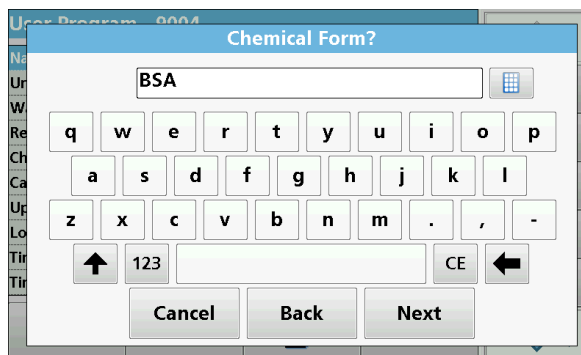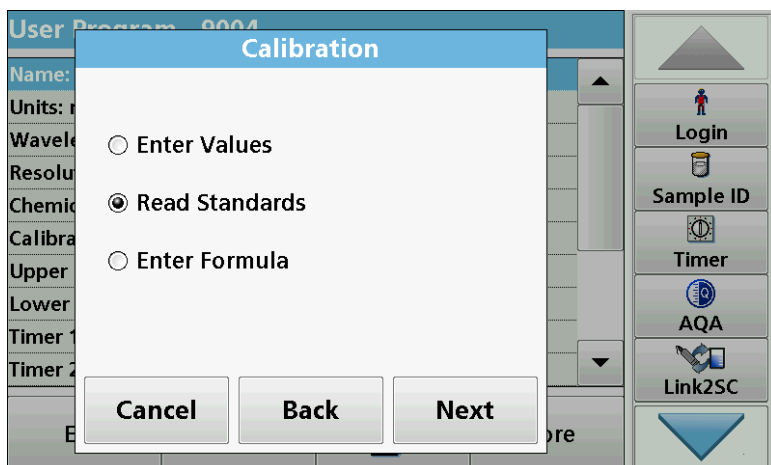
Select the **Wavelength** (here: 595 nm).

Select **Concentration Resolution**   and **Chemical Form (BSA)**

Select **Measure Standards** and measure a suitable standard series (e.g. from BSA).

Read Standards

mg/L	Abs
0.0000	0.000
4.0000	0.161
8.0000	0.330
12.000	0.492
16.000	0.660
20.000	0.823

Login
Sample ID
Timer
AQA
Trends

Cancel | Zero | Read | Next

Read Standards

C = 24.297*A

Login
Sample ID
Timer
AQA
Trends

Curve Fit r²=1.0000

Back | Next Curve | Force 0: On | Done

The calibration curve is shown. Confirm with **Done**.

The gradient found in the example (25 mg/L = 1.000 absorption) corresponds to the spectral absorption coefficient of 4.0*10-2 (mg/L final volume/µg dissolved protein per cm)

User Program - 9004

Name: Bradford
Units: mg/L
Wavelength: 595 nm
Resolution: 0.01
Chemical Form 1: BSA
Calibration: C = 0.0 + 24.297*A
Upper Limit: Off
Lower Limit: Off
Timer 1: Off
Timer 2: Off

Sample ID
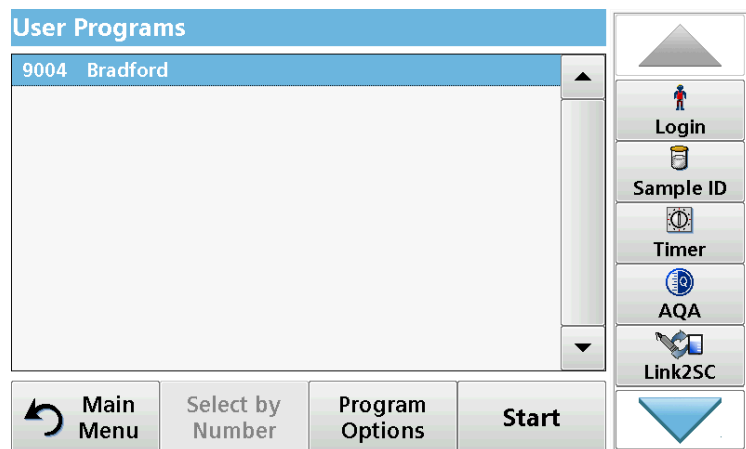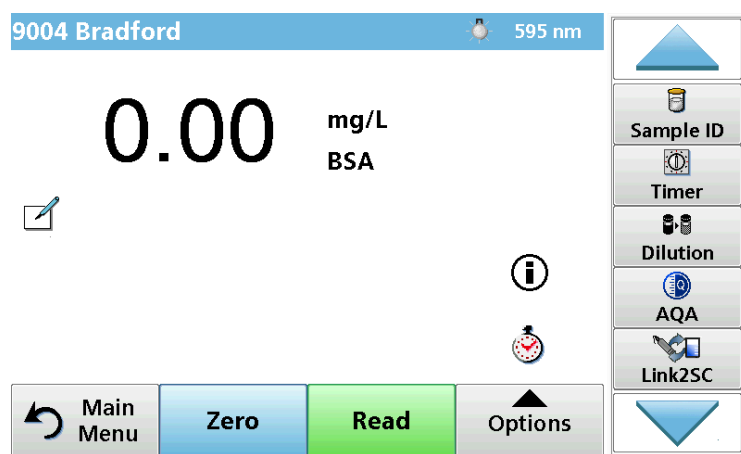Timer
AQA
Link2SC
Trends

Exit | Edit | | Store

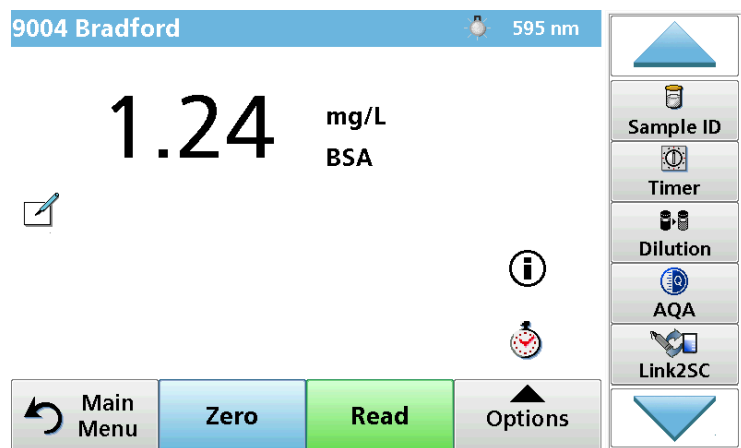Finally, the test can be saved. Press **Save**.

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Use of the User Program on the DR6000

Select **User Programs** and the corresponding program (here: program 9004 Bradford).

Start the program with the measurement of the blank value (buffer). Insert the cuvette and press **Zero**.

Then add the sample to the cuvette and measure the sample by pressing on **Read**. The result is shown in the display as **mg/L BSA**.

Reference: Bioanalytics/Friedrich Lottspeich; Haralabos Zorbas (ed.) – Heidelberg; Berlin; Spektrum Akademischer Verlag, 1998