Application Note 2014/03

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Colibri Microvolume Spectrometer

Microvolume protein quantification based on amido black (SingleQuant Assay)

Introduction

SingleQuant assay bases on the precipitation of proteins as insoluble dye complexes with acidic, ethanolic amido black 10B solution (Popov et al.). After precipitation the protein-dye complexes are spinned down. The pellet is washed and resolubilized with NaOH. The thereby released dye amount is measured at 624 nm and is proportional to the start amount of protein.

Advantages of the method:
· Precise and reproducible assay data
· Fast assay within 45 min
· No disturbance of the protein measurement by detergents or reducing reagents

Materials

- Colibri Microvolume Spectrophotometer (Titertek-Berthold)
- SingleQuant Assay Kit (SERVA Electrophoresis, Cat. No: 39226)
- Centrifuge for 1.5ml sample tubes
- Magnetic stirrer
- Vortex mixer

Methods

The SingleQuant Assay Kit is prepared after kit manufacturer instructions for using microcuvettes (300 µl).

Reference solutions with 2,4,6,8 µg of BSA and ddH_2O for blank were prepared and transferred into test tubes.
To each test tube 600 µl SingleQuant assay solution was added. After vortexing and incubation for 5 minutes the test tubes were centrifuged 5 minutes (12000xg). The supernatant was discarded and washed twice with each 600µl wash solution. To resuspend the pellet 300µl elution solution was added and the sample tubes were vortexed.

2 µl of each solution were measured in Colibri microvolume spectrometer in triplicates. Prior to measurement the blank was determined by using ddH_2O.
Measurement data was transferred to a PC and analyzed with Excel.

Colibri software settings:
Protocol: UV/VIS
Pathlength: normal (1mm)

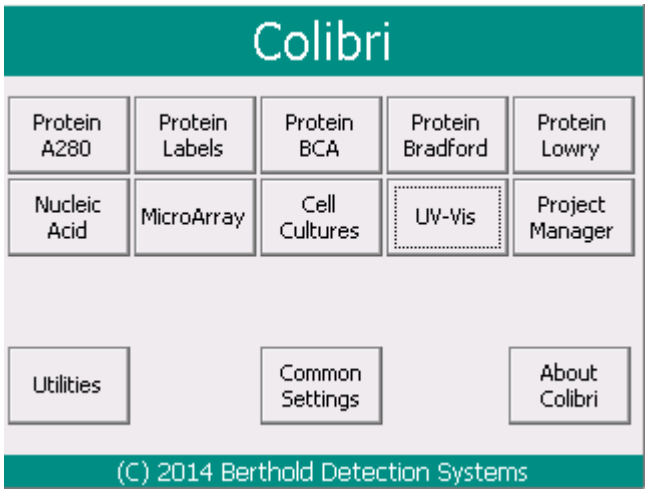

Figure 1a: Colibri main screen – select UV/VIS protocol

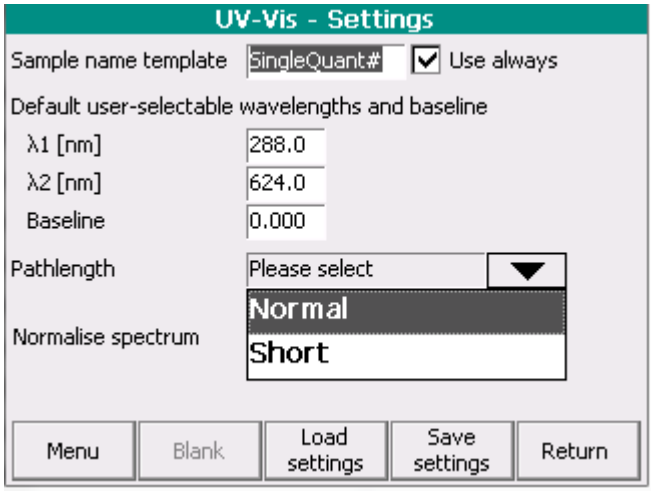

Figure 1b: select normal pathlength

Results

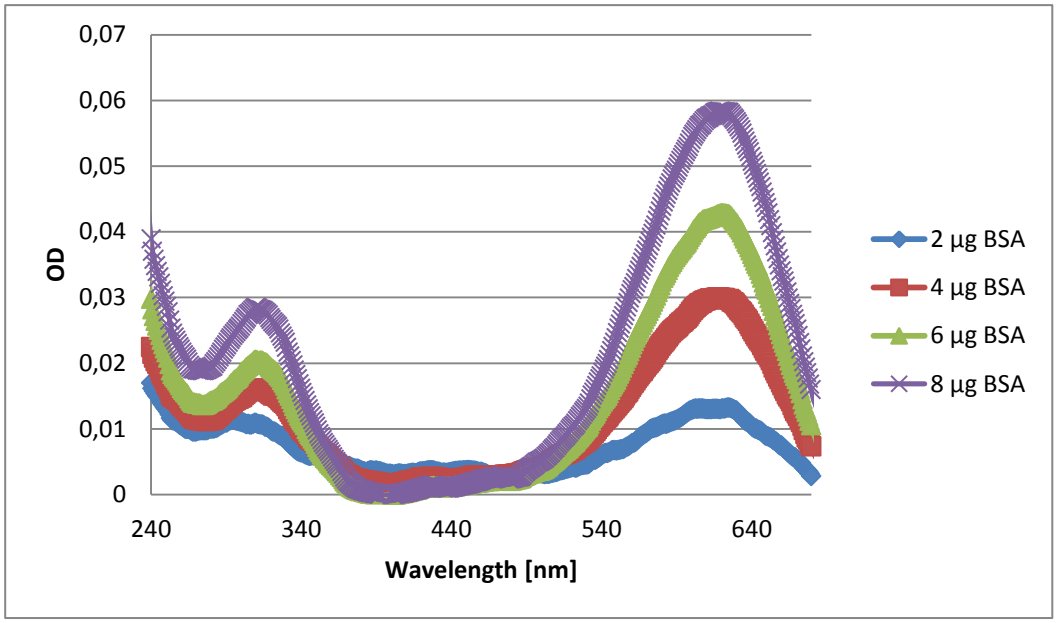

Figure 2: Complete UV/VIS spectra of BSA standards measured with Colibri microvolume spectrophotometer.

For plotting a BSA reference standard curve the measurment values at 624 nm were selected.

BSA [µg]	Measurement values at 624 nm			Mean
2	0,0132	0,0137	0,0153	0.0140
4	0,0297	0,0284	0,0273	0.0284
6	0,0427	0,0397	0,0373	0.0399
8	0,0582	0,060105	0,0657	0.0613

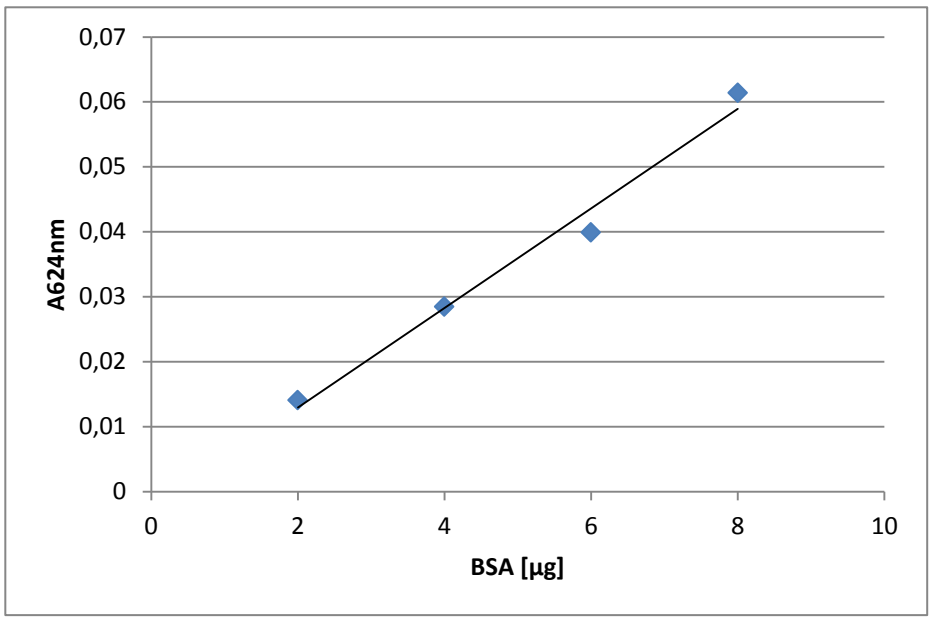

Figure 3. SingleQuant protein standard curve measured with Colibri microvolume spectrophotometer.

Conclusion:

SingleQuant assay together with Colibri microvolume spectrometer provides a convenient and fast method for protein quantitation. BSA reference standard curve shows excellent linearity.
As only 2 µl sample volume is needed for each measurement valuable sample volume and reagents can be saved.

Acknowledgement:

Samples and labspace provided by SERVA Electrophoresis. Special thanks to Dr. Seidler and Ms. Griebel.

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