INSTRUCTION MANUAL

SingleQuant Assay Kit

Kit for Protein Quantification

(Cat. No. 39226.01)

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1 . SingleQuant Assay Kit

1.1. General information

The SingleQuant assay bases on the precipitation of proteins as insoluble dye complexes with acidic, ethanolic amido black 10B solution (Popov et al. 1975). 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 procedure (ca. 45 min.)**
- **No disturbance of the protein measurement by detergents (SDS, Nonident P40, CHAPS) or reducing reagents as DTT or β-mercaptoethanol** in contrast to procedures after Lowry, Bradford or with bicinchoninic acid (BCA™). The SingleQuant protein determination is an “endpoint assay” (the absorbance of the sample remain stable over the time) and it is stable at least for 2 hours.
- **SingleQuant Assay** Kit is optimal for **single based protein quantification. For high-throughput protein quantification we recommend ProtaQuant Assay Kit (Art. No. 39225)**

Note: Ampholytes bind the dye as well and simulate too high protein concentrations. Therefore protein measurement of analytic solutions for **2D-gel electrophoresis** must **be done before ampholyte addition**.

1.2. Kit components

Reference standard (BSA) 39226.01 39226.02	 1 x 6 mg 3 x 6 mg
SingleQuant dye 39226.01 39226.02	 1x Vial 3x Vials
Washing solution 39226.01 39226.02	 1 x 400 ml 3 x 400 ml
Elution solution 39226.01 39226.02	 1 x 120 ml 1 x 360 ml

1.3. Additionally required equipment

1.3.1. General

- Eppendorf centrifuge (or equivalent device suitable for centrifugation of 1.5 ml sample tubes at 12 000xg)
- Magnetic stirrer
- Vortex mixer

1.3.2. Measurement of absorption using Microcuvettes

- Photometer suitable for measurement at 624 nm and the usage of microcuvettes
- Microcuvettes suitable for 600 µl assay volume
- Microcuvettes suitable for 300 µl assay volume

1.3.3. Measurement of absorption using Microtiter plates

- ELISA reader suitable for measurement at 624 nm (e.g. 620 nm filter)
- Microtiter plates, suitable for ELISA reader applications

1.4. Storage conditions

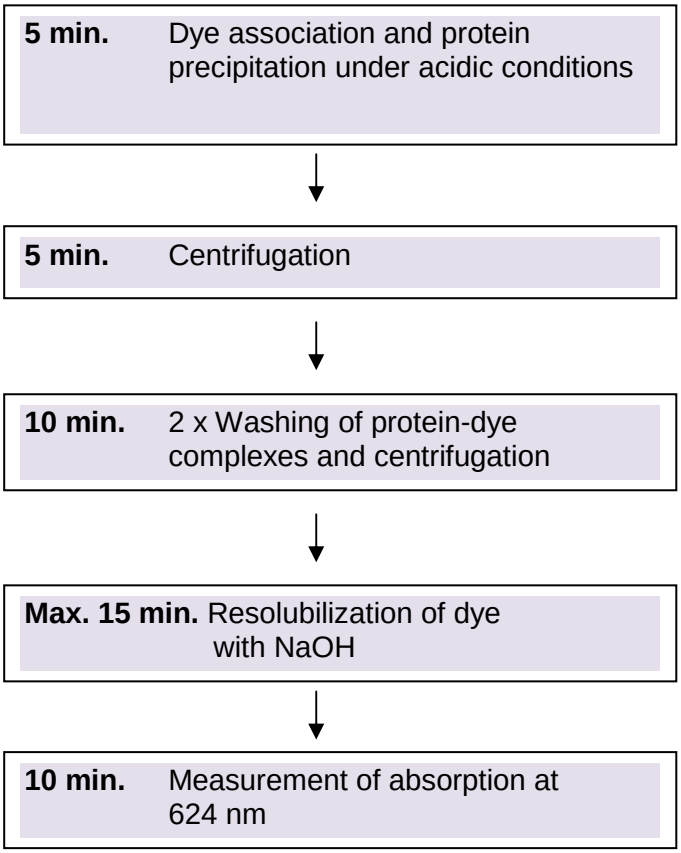
Recommended storage temperature for the unopened kit is 4 ℃.
The opened or reconstituted components should be stored as described below:

Kit component	Storage condition
Reference standard (BSA)	Aliquots; -20 ℃
Wash solution	RT
Elution solution	RT
SingleQuant stock solution	4 ℃

Under these storage conditions the kit components are at least useable until: see expiry date on package.

2. SingleQuant Assay protocol

2.1. Overview of the assay procedure



2.2. Procedure of the SingleQuant Assay

2.2.1. Preparation of solutions

SingleQuant stock solution Resolve contents of SingleQuant dye vial completely in 2.5 ml wash solution by stirring over night.

SingleQuant assay solution Dilute SingleQuant stock solution 1:50 with wash solution, mix and filter. **Prepare fresh.**

Wash solution ready-to-use

Elution solution ready-to-use

Reference standard stock solution Resolve the content of the vial in 6 ml dist. H_2O. (concentration: 1 mg/ml BSA, store aliquots at -20 °C).

Reference standard working solution Dilute 1:10 (for example fill up 70 µl of stock solution to 700 µl with dist. H_2O)

Blank solution Use as blank solution dist. H_2O or buffer in which the protein samples are solved. H_2O and buffer solution should be treated in the same way as the reference solution.

Reference solutions Prepare the BSA reference solutions according to the following schema:

No.	BSA amount	Solution quantities	
R1	2 µg	20 µl	Reference standard working sol. (conc. 0.1 mg/ml)
		600 µl	SingleQuant Assay solution
R2	4 µg	40 µl	Reference standard working sol. (conc. 0.1 mg/ml)
		600 µl	SingleQuant Assay solution
R3	6 µg	60 µl	Reference standard working sol. (conc. 0.1 mg/ml)
		600 µl	SingleQuant Assay solution
R4	8 µg	80 µl	Reference standard working sol. (conc. 0.1 mg/ml)
		600 µl	SingleQuant Assay solution

2.2.2. Procedure: Assay using Microcuvettes (300 µl assay volume) or Microtiter plates

Perform assay as triplicate determination. After each centrifugation step, we recommend to proceed immediately with the protocol, otherwise the pellet may become instable and material could be lost.

Pipet dist. H_2O and buffer for blank
reference solution and samples into test tubes

↓

Add ***600 µl SingleQuant assay solution***

↓

Close test tubes

↓

Vortex test tubes

↓

Incubate **5 min** at RT

↓

Centrifuge **5 min** at 12 000xg

↓

Discard supernatant

↓

Add ***600 µl wash solution***

↓

Vortex test tubes

↓

Centrifuge **5 min** at 12 000xg

↓

Discard supernatant

↓

Add ***600 µl wash solution***

↓

Vortex test tubes

↓

Centrifuge **5 min** at 12 000xg

↓

Discard supernatant

↓

To remove supernatant completely, invert the test tube on a filter paper and dry seam of the vial

↓

Add ***300 µl elution solution***

↓

Resuspend entire pellet by vortexing

If resuspension is incomplete, incubate 1**5 min** ***at 40 ℃***

↓

Transfer the solution in cuvettes or microtiter plate

Measure absorbance at 624 nm (A_{624nm})

2.2.3. Procedure: Assay using Microcuvettes (600 µl assay volume)

Perform assay as triplicate determination. After each centrifugation step, we recommend to proceed immediately with the protocol, otherwise the pellet may become instable and material could be lost.

Pipet dist. H_2O and buffer for blank
reference solution and samples into test tubes

↓

Add ***600 µl SingleQuant assay solution***

↓

Close test tubes

↓

Vortex test tubes

↓

Incubate **5 min** at RT

↓

Centrifuge **5 min** at 12 000xg

↓

Discard supernatant

↓

Add ***600 µl wash solution***

↓

Vortex test tubes

↓

Centrifuge **5 min** at 12 000xg

↓

Discard supernatant

↓

Add ***600 µl wash solution***

↓

Vortex test tubes

↓

Centrifuge **5 min** at 12 000xg

↓

Discard supernatant

↓

To remove supernatant completely, invert the test tube on a filter paper and dry seam of the vial

↓

Add ***600 µl elution solution***

↓

Resuspend entire pellet by vortexing

If resuspension is incomplete, incubate 15 min at 40 °C

↓

Transfer solution in cuvettes
Measure absorbance at 624 nm (A_{624nm})

2.2.4. Calculation of protein concentrations

Create a table with the absorbance results obtained from the assay. From the values obtained for the BSA reference solutions create a calibration curve, which is used to determine the protein concentration in the unknown sample.

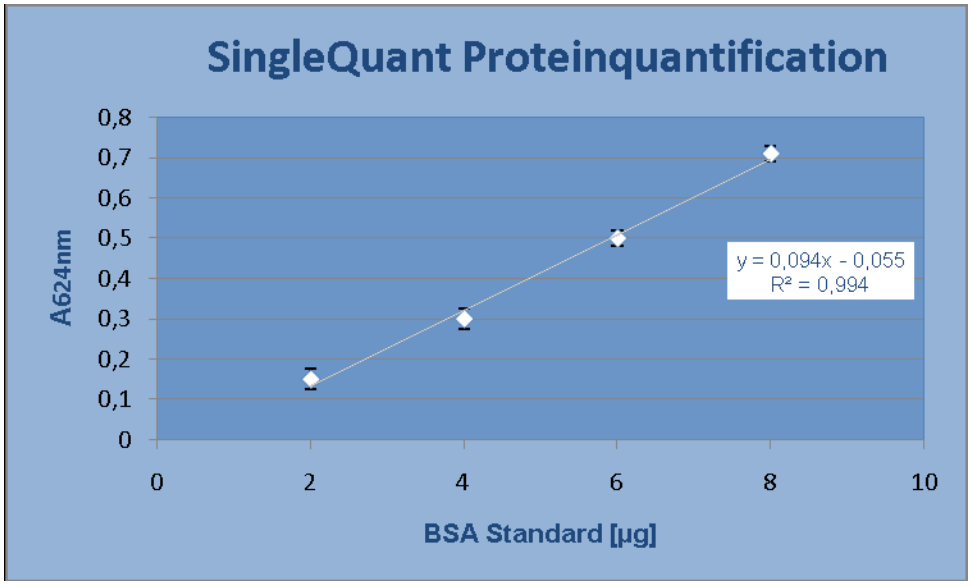
Table 1: Example of assay data table of BSA reference solutions

Assay values A_{624nm}	BSA amount [µg]
0.17	2
0.12	2
0.15	2
0.31	4
0.32	4
0.27	4
0.52	6
0.48	6
0.50	6
0.69	8
0.71	8
0.73	8

Table 1 shows exemplary absorbance results for creation of the BSA calibration curve (samples were solved in water) and **graph 1** the consequential resulting calibration curve.

Note:
The data below should not be used as a replacement of a calibration curve. The absorbance of the BSA reference solutions in each assay will differ from those presented here.

Graph 1: BSA calibration curve produced from the mean value of the assay data from the table 1.



The SingleQuant protein standard curve was produced using BSA as a standard in triplicate points. Standard deviation for each value is depicted on the graph.
The data are fit with a linear regression by the line y=0.094x-0.055 with an R^2 value of 0.994.

The calculation is made with linear regression of the reference solutions and the following conversion of the absorption values of the sample solutions in protein concentrations through the regression equation.

3. Literature

- **Schaffner W., Weissmann C.,** A rapid, sensitive, and specific method for the determination of protein in dilute solution, Anal. Biochem**.** (1973); **65:** 502-514.

- **Popov N., Schmitt M., Schulzeck S., Matthies H.,** Reliable micro method for determination of the protein content in tissue homogenates, Acta Biol. Med. Ger. (1975); **34 (9)**: 1441-1446.