INSTRUCTION MANUAL

# ProtaQuant Assay Kit

**Kit for protein quantification**
**(Cat. No. 39225.01)**

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# Contents





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# 1. ProtaQuant Assay Kit

## 1.1. General information

The ProtaQuant™ Assay bases on the precipitation of proteins as insoluble dye complexes with acidic, methanolic 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 620 nm and is proportional to the start amount.

**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 bicinchonic acid (BCA™).
- **Format** of the **ProtaQuant Assay** is optimal for **high throughput procedures**

**Note:** Ampholytes bind the dye as well and therefore 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

| | |
|---|---|
| 10 x Dye concentrate | 2,5 ml |
| Elution solution (0.1 M NaOH) | 30 ml |
| Bovine serum albumin | 15 mg |
| Multiscreen HTS™-DV 96-well Filtration System, clear styrene | 1 |
| PP-Masterblock 2 ml, 96 well | 1 |
| Microplate 96Deep-well PP 350 µl square well | 1 |
| 96 F Microwell plate | 1 |


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## 1.3. Additionally required chemicals and equipment

- Sorvall centrifuge Multifuge 3 S-R and rotor 75006444 (or equivalent device)
- Microtiter plate reader
- Microtiter plate shaker
- Magnet stirrer
- Vortex mixer

**Materials:**

- Glacial acetic acid
- Methanol, Cat. No. 45630

## 1.4. Storage conditions

The solutions of the ProtaQuant Assay Kit as well as the BSA reference standard are stored at 2 °C – 8 °C. The remaining kit components are stored at room temperature. If stored at the recommended temperature at least usable until: see expiry date on package.


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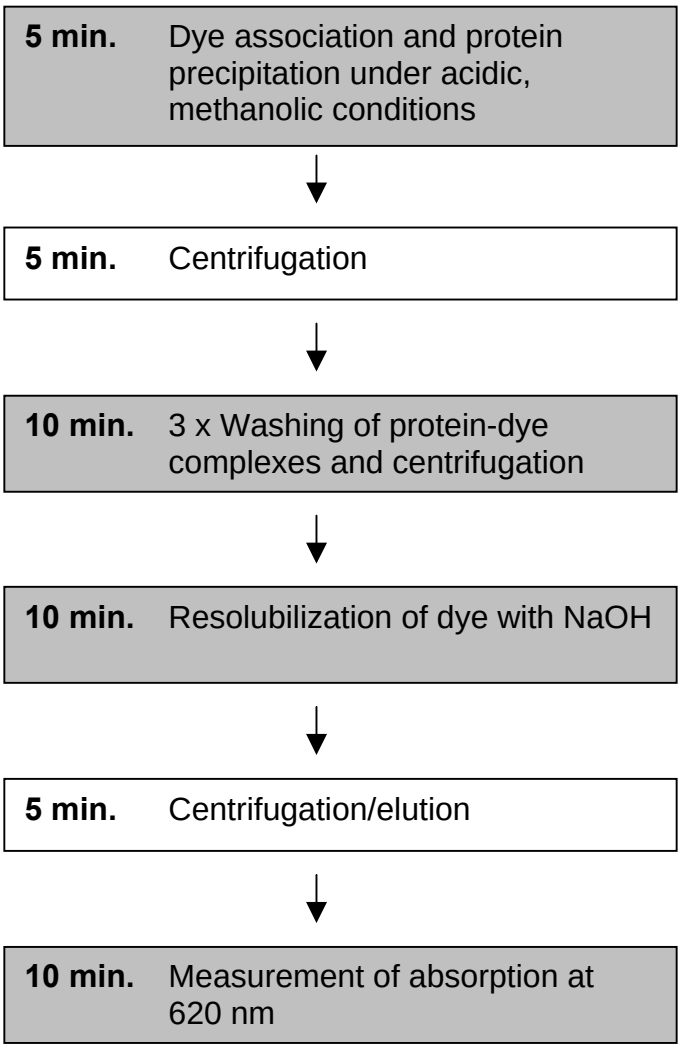
# 2. ProtaQuant Assay protocol

## 2.1. Overview of the assay procedure



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## 2.2. Procedure of the ProtaQuant Assay

### 2.2.1. Preparation of solutions

**Wash solution** Add 15 ml glacial acetic acid to 135 ml methanol in an 250 ml-measuring cylinder.

**10 x Dye concentrate** ready to use

**Dye solution** Fill up with wash solution the complete volume of 10 x dye concentrate (2.5 ml) in an 25 ml-cylindrical sensing chamber to 25 ml end volume.

**Elution solution** ready to use

**BSA stock solution** Solve contents of the vial with 3 ml sample buffer (buffer, in which the sample to be quantified is solved; concentration: 5 mg/ml).

**Blank solution** sample buffer (R8)

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

| No. | Concentration of reference solutions | Solution quantities | |
|---|---|---|---|
| R1 | 1.75 mg/ml | 350 µl | BSA stock solution (conc. 5 mg/ml) |
| | | 650 µl | sample buffer |
| R2 | 1.5 mg/ml | 300 µl | BSA stock solution (conc. 5 mg/ml) |
| | | 700 µl | sample buffer |
| R3 | 1.25 mg/ml | 250 µl | BSA stock solution (conc. 5 mg/ml) |
| | | 750 µl | sample buffer |
| R4 | 1 mg/ml | 200 µl | BSA stock solution (conc. 5 mg/ml) |
| | | 800 µl | sample buffer |
| R5 | 0.75 mg/ml | 150 µl | BSA stock solution (conc. 5 mg/ml) |
| | | 850 µl | sample buffer |
| R6 | 0.5 mg/ml | 100 µl | BSA stock solution (conc. 5 mg/ml) |
| | | 900 µl | sample buffer |
| R7 | 0.25 mg/ml | 50 µl | BSA stock solution (conc. 5 mg/ml) |
| | | 950 µl | sample buffer |
| R8 | 0 mg/ml | - | BSA stock solution (conc. 5 mg/ml) |
| | | 1000 µl | sample buffer |


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### 2.2.2. Procedure

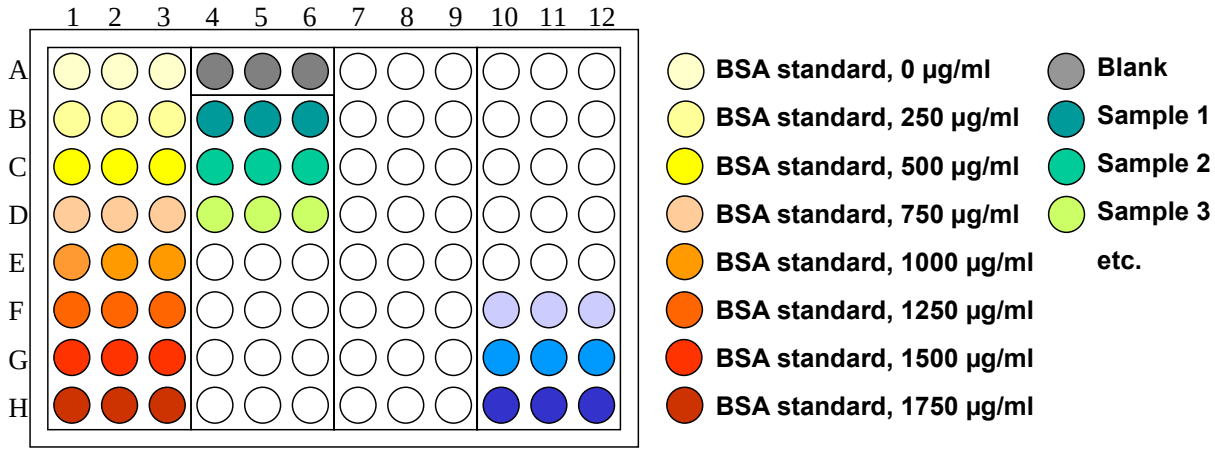


**Fig. 1:** Recommended layout for 1 - 23 samples in triple measurement

1. Place **Multiscreen HTS™-DV 96-well Filtration System** plate onto **PP-Masterblock 2-ml**.

2. Pipet **30 µl sample buffer** into wells of **Multiscreen HTS™-DV 96-well Filtration System** plate.

3. Add **20 µl protein solution** (reference- or sample solution) **in sample buffer**.

4. Give **180 µl dye solution** to each well. From now on stop time, and incubate for **30 sec. at 300 rpm on a shaker** and thereafter **until 5 minutes are elapsed** without shaking at room temperature.

5. **Centrifuge** both plates together for **5 minutes at 4000 rpm (3000 x g)** (it is not necessary to remove the filtrate from the lower PP-Masterblock plate, because the wells of the plate can hold a total volume of up to 2 ml, and therefore as well the solutions of the washing steps).

6. Add **250 µl washing solution** to each well for removing of excessive dye. Centrifuge for **3 minutes at 4000 rpm (3000 x g)**.

7. Repeat **step 6 twice**.

8. Place the **Multiscreen HTS™-DV 96-well Filtration System** plate for elution of the dye onto the **96Deep-well PP 350 µl square well microplate**. Resolubilize the dye by addition of **250 µl elution solution** to each well. Support the resolubilization by **shaking** of plate for **10 minutes at 200 rpm**.

9. **Centrifuge** plates for **4 minutes at 4000 rpm (3000 x g)**.

10. Transfer **200 µl eluate** and additionally **200 µl elution solution** as blank with a multichannel pipette into a 96-well plate.

11. Measure **absorption at 620 nm** in a microtiter plate reader.



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### 2.2.3. 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.

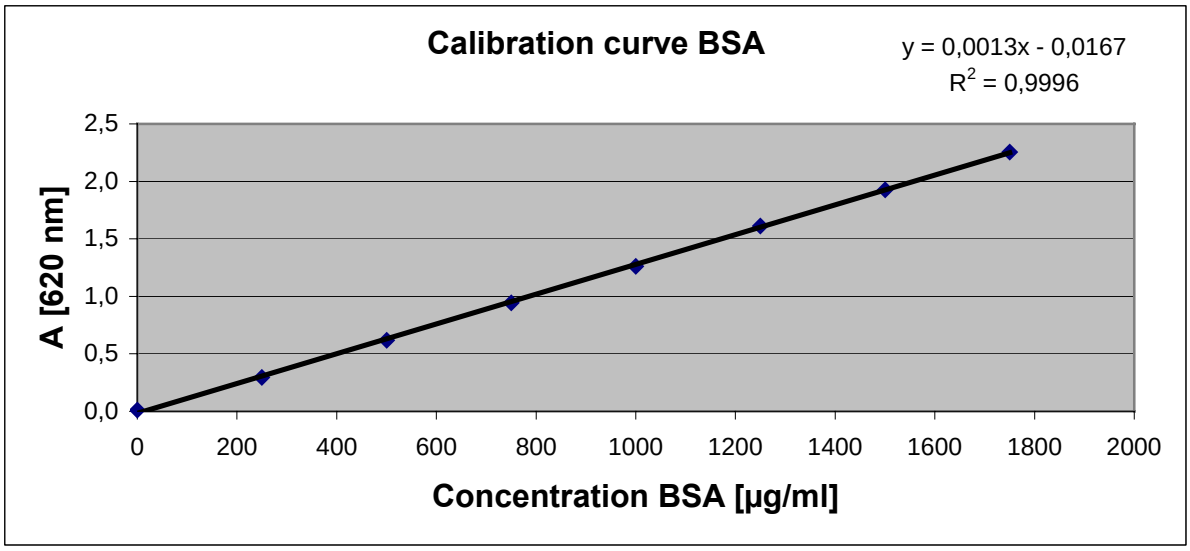
**Table 1** shows exemplary absorbance results for creation of the BSA calibration curve (samples were solved in water) and **graph 1** the hence resulting calibration curve.

**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.

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

| Concentration | Assay values $A_{620}$ | Assay values minus blank | Calculated protein concentration [µg/ml] | SD assay values | CV in % assay values |
|---|---|---|---|---|---|
| Blank | 0,0304 | | | 0,000 | 0,987 |
| 0 | 0,0415 | 0,0111 | 21,49 | 0,009 | 21,386 |
| 250 | 0,3259 | 0,2955 | 241,19 | 0,013 | 4,114 |
| 500 | 0,6478 | 0,6174 | 489,97 | 0,013 | 1,991 |
| 750 | 0,9741 | 0,9437 | 742,07 | 0,016 | 1,675 |
| 1000 | 1,2926 | 1,2622 | 988,17 | 0,039 | 3,034 |
| 1250 | 1,6428 | 1,6124 | 1258,77 | 0,028 | 1,675 |
| 1500 | 1,9579 | 1,9275 | 1502,24 | 0,037 | 1,912 |
| 1750 | 2,2864 | 2,2560 | 1756,10 | 0,052 | 2,280 |

**Graph 1: BSA calibration curve produced from assay data of table 1**




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.



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## 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.




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