Bioanalysis

MACHEREY-NAGEL

User manual

Determination of protein concentration

■ Protein Quantification Assay

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1 Components

1.1 Kit contents

	Protein Quantification Assay	
REF	**50 assays 740967.50**	**250 assays 740967.250**
Protein Solving Buffer PSB	7.5 mL	40 mL
BSA (Bovine Serum Albumin; reference protein)*	1 mg	2 × 1 mg
Quantification Reagent QR	20 mL	20 mL
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1.2 Consumables and equipment to be supplied by user

Consumables

- Microplates, flat-bottom (e.g., UV-Star Microtiter plate, 96-well, F-bottom, Greiner bio-one REF 655801; similar non-UV transparent microtiter plates are also suitable) or semi-micro cuvettes (e.g., Plastibrand 1.5 mL semi-micro disposable cuvettes, Brand REF 759115) or micro-cuvettes (e.g., Plastibrand UV-Cuvette micro, Brand, REF 759220).
- 1.5 mL microcentrifuge tubes (to prepare dilution series for the calibration curve and to set up reactions when following the semi-micro cuvette assay procedure)
- Disposable pipette tips

Equipment

- Manual pipettors
- Centrifuge for microcentrifuge tubes (to clean microcentrifuge lids if necessary)
- Vortex mixer
- Mixer or shaker for microplates
- Photometer set to 570 nm (570 nm is recommended, other wavelength settings in the range of 530 – 700 nm are also suitable), either for microplates (microplate assay procedure), for semi-micro/microcuvettes (semi-micro cuvette and/micro cuvette assay procedure) or for low volume analysis (e.g., NanoDrop (Thermo Scientific), NanoVue (GE Healthcare), or NanoPhotometerTM (Implen)).
- Personal protection equipment (e.g., lab coat, gloves, goggles)

* For preparation of working solutions and storage conditions see section 3.

1.3 About this user Manual

It is recommended to read the instructions of this user manual carefully before use. All technical literature is also available on the Internet at ***www.mn-net.com***.

Please contact Technical Service regarding information about changes of the current user manual compared to previous or updated revisions.

2 Product description

2.1 The basic principle

The **Protein Quantification Assay** is a convenient and reliable kit for the determination of protein concentration in samples typically used for SDS-PAGE (sodium dodecylsulfate polyacrylamide gel electrophoresis). It is mainly designed for proteins solved in Protein Solving Buffer PSB (components of NucleoSpin® RNA/Protein and NucleoSpin® TriPrep and Protein Solving Buffer Set), but will also work with proteins solved in buffer as described by Laemmli (1970), or similar. These protein sample buffers usually contain SDS, a reducing agent, dye, and a component to increase the buffer density. The majority of protein quantification assays* are either influenced by or incompatible with SDS, reducing agents, or dyes commonly present in protein sample buffers. The **Protein Quantification Assay** however, is well suited for such buffer systems. It is a fast and sensitive assay, based on a modification of a protocol described by Karlsson et al. (1994). The samples are mixed with Protein Solving Buffer PSB and subsequently incubated for 30 minutes with Quantification Reagent QR. After incubation light extinction is measured photometrically. Light extinction is caused by turbidity appearing after addition of Quantification Reagent QR. The protein concentration is determined in reference to a BSA (Bovine Serum Albumin) calibration curve (BSA is provided with the **Protein Quantification Assay**).

2.2 Kit specifications

- **Protein Quantification Assay** allows the determination of protein concentration in samples containing up to 10 % SDS and comprising reducing agent (e.g., ß-mercaptoethanol (BME), dithiothreitol (DTT), dithioerythritol (DTE) or tris- (2-carboxyethyl) phosphine hydrochloride (TCEP)), buffering salts (e.g., TRIS or BIS-TRIS), dye (bromphenol blue), and a component to create a high density of the solution (e.g., glycerol or sucrose).
- **Protein Quantification Assay** is designed for the determination of protein concentration in samples with low nucleic acid concentration, as obtained with NucleoSpin® RNA/Protein or NucleoSpin® TriPrep. For samples rich in nucleic acids the quantification is less accurate.

* For example: Coomassie Brilliant Blue G-250, Bradford 1979; copper tartrate solution and Folin reagent, Lowry *et* al. 1951; Cu2+/Cu1+ - BCA interaction, Smith et al. 1985.

- **Protein Quantification Assay** is suited for samples comprising protein solved in buffers, commonly used for SDS-PAGE (e.g., Laemmli buffer). Accuracy depends on nucleic acid content of the sample. For typical cultured cells (e.g., HeLa) accuracy is affected by approximatively 5 – 20 % due to nucleic acid content*.
- The kit REF **740967.50** is sufficient for 50 protein determinations plus six calibration curves with seven calibration points each (approx. 100 reactions in total), according to the microplate assay. Alternatively the kit is sufficient for approx. 10 reactions according to the semi-micro cuvette assay (three protein determinations plus seven calibration points).
- The kit REF **740967.250** is sufficient for 250 protein determinations plus 25 calibration curves with seven calibration points each (approx. 450 reactions in total), according to the microplate assay. Alternatively, the kit is sufficient for approx. 50 reactions according to the semi-micro cuvette assay (26 protein determinations plus three calibration curves with seven calibration points each).
- Following the **microplate assay procedure** the kit allows the determination of protein amount (exemplary BSA) in the range of 0.6 – 20 µg per assay provided in a standard volume of 20 µL Protein Solving Buffer PSB (alternatively 1 – 60 µL). This corresponds to a protein concentration of 30 – 1000 ng/µL. This concentration range can be expanded to 10 – 20,000 ng/µL if alternative sample volumes (1 – 60 µL) are applied.
- Following the **semi-micro cuvette assay procedure** the kit allows the determination of protein (exemplary BSA) amount in the range of 6 – 200 µg per assay provided in a standard volume of 200 µL Protein Solving Buffer PSB. This corresponds to a protein concentration of 30 – 1000 ng/µL.

* One microgram DNA causes ca. 50 – 70 % of the extinction signal caused by one microgram protein (BSA). One microgram RNA causes ca. 10 – 40 % of the extinction signal caused by one microgram protein (BSA).

DNA, RNA, and protein content of a typical cell and influence on the protein quantification:

Molecule	Content per cell	Content per one million cells	Extinction signal obtained with the Protein Quantification Assay relative to the reverence protein BSA	Extinction signal obtained relative to total protein
DNA	6 pg	6 µg	~ 50 – 70 %	3 – 5 %
RNA	10 – 30 pg	10 – 30 µg	~ 10 – 40 %	1 – 12 %
Protein	100 – 200 pg	100 – 200 µg	~ 100 %	100 %
				Total: 104 – 117 %

Signal obtained from total cell extract containing RNA and DNA, relative to nucleic acid free total protein: 104 – 117 %.

Table 1: Kit specifications at a glance*

	Protein Quantification Assay
Sample size	1 – 600 µL containing 0.6 – 200 µg protein (BSA equivalents)
Microplate assay	0.6 – 20 µg protein (BSA equivalents) in 20 µL, corresponding to 30 – 1000 ng/µL
Semi-micro cuvette assay	6 – 200 µg protein (BSA equivalents) in 200 µL, corresponding to 30 – 1000 ng/µL
Sample type	Protein solved in Protein Solving Buffer PSB, Laemmli buffer or equivalent, preferable free of nucleic acids
Protein concentration	Approx. 30 – 1,000 ng/µL (standard range) or Approx. 10 – 20,000 ng/µL (extended range)
Correlation coefficient	0.97 – 1.00
Wavelength for light extinction measurement	570 nm (530 – 700 nm)
Time	Approx. 40 min
Use	For research use only

* Kit specifictions vary depending on the type of assay. Please find more detailed information in the tables below:

Type of assay	Required sample volume	Protein amount per assay	Determinable protein concentration
Microplate	20 µL (1 – 60 µL)	0.6 – 20 µg	30 – 1000 ng/µL
Semi-micro cuvette	200 µL (10 – 600 µL)	6 – 200 µg	30 – 1000 ng/µL
Micro cuvette	40 µL (1 – 120 µL)	1.2 – 40 µg	30 – 1000 ng/µL
Low volume	7.5 µL	0.47 – 7.5 µg	60 – 1000 ng/µL

	740967.50			**740967.250**		
Type of assay	Protein determination	Calibration curves (7 points per curve)	Total number of reactions	Protein determination	Calibration curves (7 points per curve)	Total number of reactions
Microplate	50	6	Approx. 100	250	25	Approx. 450
Semi-micro cuvette	3	1	Approx. 10	26	3	Approx. 50
Micro cuvette	35	3	Approx. 55	130	15	Approx. 235
Low volume	800	25	Approx. 1000	3000	70	Approx. 3500

2.3 Handling, preparation, and storage of starting materials

After dissolving protein in Protein Solving Buffer PSB (with or without Reducing Agent TCEP), Laemmli buffer, or analogs, freeze your protein samples for long term storage or keep samples at 4 °C for short term storage. Before use, make sure that the samples are free of precipitates. If necessary heat to approximately 30 °C in order to dissolve any possible SDS precipitate. Subsequently, spin sample briefly to remove any further insoluble matter.

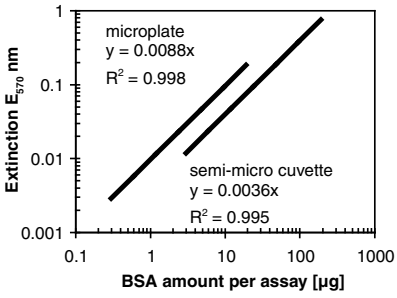
Protein samples obtained with NucleoSpin® RNA/Protein or NucleoSpin® TriPrep and dissolved in Protein Solving Buffer PSB (with or without Reducing Agent TCEP), are optimal for determination of protein concentration with the **Protein Quantification Assay**.

Quantification of protein samples obtained by boiling cells or tissue directly in PSB (with or without Reducing Agent TCEP), Laemmli buffer, or analogs is possible, but the measurement may be less accurate due to the presence of nucleic acids, which interfere with the assay. The extent of interference depends on the content of protein and nucleic acid in the sample. Many samples, like, for example, cultured HeLa cells or liver tissue, contain much more protein than nucleic acid and thus nucleic acids cause only small interference (see footnote page 6).

Wear gloves at all times during the handling to reduce risk of sample contamination with skin keratins.

2.4 Calibration curves

Reference protein (BSA) dilution series give good correlations with measured light extinction. Typical correlation coefficients of 0.97 – 1.00 are obtained in the range of approx. 0.03 – 1 µg/µL BSA concentration. BSA concentration versus extinction and BSA amount versus extinction are shown in Figure 1.



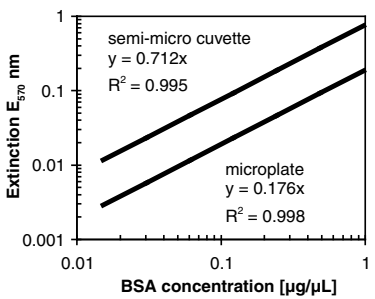


Figure 1 Correlation between BSA amount and extinction signal as well as between BSA concentration and extinction signal.
For the microplate assay BSA was supplied in 20 µL; path length for extinction measurement was 3 mm. For the semi-micro cuvette assay BSA was supplied in 200 µL; path length for extinction measurement was 10 mm.

2.5 Recommended sample volumes

As guidance, follow the recommendations of Table 2–Table 5 to choose an appropriate volume of your sample for measuring. For the initial determination of protein concentration in samples containing hard-to-estimate protein amounts, measurement of multiple sample volumes (e.g., 2 µL, 5 µL, 50 µL) is recommended. This will increase the probability that one of the measured protein amounts lies within the range of the calibration curve.

For protein samples obtained with NucleoSpin® RNA/Protein or NucleoSpin® TriPrep, see the respective user manual for a first estimation of the protein yield.

Table 2: Microplate assay – Recommended sample volumes for protein quantification

Expected protein concentration	Recommended sample volume	Protein amount per well
0.01 – 0.33 µg/µL	60 µL	0.6 – 20 µg
0.03 – 1.0 µg/µL	20 µL	0.6 – 20 µg
0.6 – 20 µg/µL	1 µL	0.6 – 20 µg

Table 3: Semi-micro cuvette assay – Recommended sample volumes for protein quantification

Expected protein concentration	Recommended sample volume	Protein amount
0.01 – 0.33 µg/µL	600 µL	6 – 200 µg
0.03 – 1.0 µg/µL	200 µL	6 – 200 µg
0.6 – 20 µg/µL	10 µL	6 – 200 µg

Table 4: Microcuvette assay – Recommended sample volumes for protein quantification

Expected protein concentration	Recommended sample volume	Protein amount per microcuvette
0.01 – 0.33 µg/µL	120 µL	1.2 – 40 µg
0.03 – 1.0 µg/µL	40 µL	1.2 – 40 µg
1.2 – 40 µg/µL	1 µL	1.2 – 40 µg

Table 5: Low volume assay – Recommended sample volumes for protein quantification

Expected protein concentration	Recommended sample volume	Protein amount per microcuvette
0.06 – 1 µg/µL	7.5 µL	0.47 – 7.5 µg

2.6 Alternative wavelengths for extinction measurement

A wavelength in the range of 530 – 700 nm is recommended for light extinction measurements. Figure 2 shows the dependency of correlation coefficient on the wavelength, used for light extinction measurement.

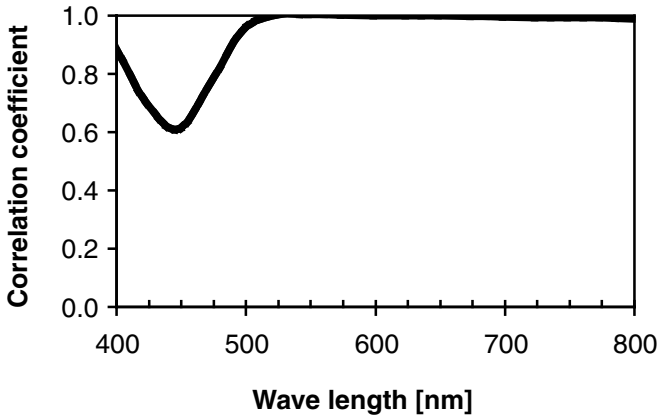


Figure 2 Dependency of correlation coefficient on the wavelength, used for extinction measurement.
Light extinction of BSA samples in the range of 0.3 – 20 µg was measured for wavelength between 400 nm and 800 nm. The correlation coefficient was calculated from the BSA amount per assay (0.3 – 20 µg per assay) and corresponding extinction signal.

3 Storage conditions and preparation of working solutions

Attention:

Quantification Reagent QR contains hydrochloric acid. Wear gloves and goggles!

- All kit components should be stored at 15 – 25 °C. Storage at lower temperatures may cause precipitation in the Protein Solving Buffer PSB. Kit components are stable up to one year.

Before starting the **Protein Quantification Assay** prepare the following:

- Dissolve the reference protein (BSA, 1 mg) in 1 mL Protein Solving Buffer PSB to obtain a 1 mg/mL BSA stock solution. Freeze BSA stock solution for storage. After thawing, keep solution at 4 °C or on ice before/after usage. If necessary, dissolve any precipitate by heating the reference solution (approx. 30 °C) before use. BSA stock solution (1 mg/mL BSA in PSB) is stable at - 20 °C for six months.

	Protein Quantification Assay	
REF	**50 assays** **740967.50**	**250 assays** **740967.250**
BSA (reference protein)	1 mg add 1 mL PSB	2 × 1 mg add 1 mL PSB to each vial

4 Safety instructions

When working with the **Protein Quantification Assay** kit wear suitable protective clothing (e.g., lab coat, disposable gloves, and protective goggles). For more information consult the appropriate Material Safety Data Sheets (MSDS available online at ***www.mn-net.com/msds***).

The waste generated with the **Protein Quantification Assay** kit has not been tested for residual infectious material. A contamination of the liquid waste with residual infectious material is highly unlikely due to strong denaturing lysis buffer but it cannot be excluded completely. Therefore, liquid waste must be considered infectious and should be handled and discarded according local safety regulations.

4.1 Disposal

Dispose hazardous, infectious or biologically contaminated materials in a safe and acceptable manner and in accordance with all local and regulatory requirements.

5 Protocols

5.1 Microplate assay procedure

Before starting the preparation:

- Check if the BSA reference protein stock solution was prepared according to section 3.
- Make sure that there are no precipitates in Protein Solving Buffer PSB and in the reverence protein (BSA) solution (if necessary, heat to approx. 30 °C).

1 Prepare a BSA (reference protein) dilution series

Number seven reaction tubes according to column A (see table below; #1: BSA stock solution).

Add **50 µL Protein Solving Buffer PSB** to tubes #2–#7 (column B).

Add **BSA solution** to tubes #2–#6 *according to column C*.

The resulting protein concentration and amount are shown in columns D and E.

PSB contains detergent! When pipetting BSA and PSB solutions avoid bubble formation and foaming as far as possible.

A	B	C	D	E
Tube	**Add PSB to tube**	**Add BSA solution to tube**	**Resulting BSA concentration**	**Resulting BSA in 20 µL**
#1	BSA stock solution		1 µg/µL	20 µg
#2	50 µL	50 µL from tube #1	0.5 µg/µL	10 µg
#3	50 µL	50 µL from tube #2	0.25 µg/µL	5 µg
#4	50 µL	50 µL from tube #3	0.125 µg/µL	2.5 µg
#5	50 µL	50 µL from tube #4	0.063 µg/µL	1.25 µg
#6	50 µL	50 µL from tube #5	0.031 µg/µL	0.625 µg
#7	50 µL	–	0 µg/µL	0 µg

The prepared BSA dilutions series is sufficient for the determination of **two** calibration curves. Freeze BSA stock solution for storage. Keep dilution series on ice during use and dispose all dilutions at the end of a working day.

2 Dispense dilution series into microplate — **20 µL of dilution series**

Add **20 µL** of **each dilution series solution** (#1–#7) into microplate wells.

(#1: BSA stock solution; #2–#6: BSA dilutions; #7: BSA-free PSB)

3	**Dispense your protein samples** Pipette **20 µL** of your **samples** to empty wells. Alternatively, 1 – 60 µL of sample can be applied.	**20 µL of samples**
4	**Fill up dilution series and protein samples** Add **40 µL PSB** to each well (dilution series and protein samples). Final volume is 60 µL. *Alternatively, when applying other sample volumes than 20 µL in step 3, fill up with PSB to a final volume of 60 µL (e.g., 10 µL sample + 50 µL PSB).*	**+ 40 µL PSB**
5	**Add Quantification Reagent QR** Add **40 µL Quantification Reagent QR** to each well (dilution series and protein samples). **Shake microplate** until a complete color change from blue to yellow occurs. *Caution: Quantification Reagent QR contains hydrochloric acid. Wear protective clothing and goggles.*	**+ 40 µL QR** **Shake microplate**
6	**Incubate** Incubate microplate for **30 min** at room temperature. Gently shake microplate after incubation, but avoid bubble formation and foaming. For optimal measurement the solution surface in the microplate well should be free of bubbles and foam. Light scattering caused by foam has impact on the measurement. *A variation in incubation time may result in reduced signal and loss of sensitivity. An incubation time of 30 ± 5 min is recommended.*	**Incubate 30 min**
7	**Measure light extinction** Measure light extinction photometrically at **570 nm**. *Light extinction can be measured in the range of 530 – 700 nm. Typical correlation coefficients (concentration of BSA versus extinction value) of 0.97 – 1.00 are obtained within this wavelength range.*	**Measure extinction at 570 nm**
8	**Calculate protein concentration** Calculate protein concentration of samples in relation to the BSA dilution series. Make sure that the protein concentration of your sample lies within the range of the largest (#1) and the smallest (#6) concentration of the calibration curve in order to obtain valid measurements. Do not extrapolate beyond this range.	**Calculate protein concentration**

5.2 Semi-microcuvette assay procedure

Before starting the preparation:

- Check if the BSA reference protein stock solution was prepared according to section 3.
- Make sure that there are no precipitates in Protein Solving Buffer PSB and in the reverence protein (BSA) solution (if necessary, heat to approx. 30 °C).

1 Prepare a BSA (reference protein) dilution series

Number seven reaction tubes according to column A (see table below; #1: BSA stock solution).

Add **250 µL Protein Solving Buffer PSB** to tubes #2–#7 (column B).

Add **BSA solution** to tubes #2–#6 according to column C.

The resulting protein concentration and amount are shown in columns D and E.

A Tube	B Add PSB to tube	C Add BSA solution to tube	D Resulting BSA concentration	E Resulting BSA in 20 µL
#1	BSA stock solution		1 µg/µL	200 µg
#2	250 µL	250 µL from tube #1	0.5 µg/µL	100 µg
#3	250 µL	250 µL from tube #2	0.25 µg/µL	50 µg
#4	250 µL	250 µL from tube #3	0.125 µg/µL	25 µg
#5	250 µL	250 µL from tube #4	0.063 µg/µL	12.5 µg
#6	250 µL	250 µL from tube #5	0.031 µg/µL	6.25 µg
#7	250 µL	–	0 µg/µL	0 µg

The prepared BSA dilutions series is sufficient for the determination of **one** calibration curve. Freeze BSA stock solution for storage. Keep dilution series on ice during use and dispose all dilutions at the end of a working day.

2 Dispense dilution series into microcentrifuge tubes — **200 µL of dilution series**

Pipette **200 µL** of **each dilution series solution** (#1–#7) into 1.5 mL microcentrifuge tubes (not supplied).

(#1: BSA stock solution; #2–#6: BSA dilutions; #7: BSA-free PSB)

3 Dispense your protein samples — **200 µL of samples**

Pipette **200 µL** of your samples to (new) microcentrifuge tubes.

Alternatively, 10 – 600 µL of sample can be applied.

4	**Fill up dilution series and protein samples** Add **400 µL PSB** to each microcentrifuge tube (dilution series and protein samples). Final volume is 600 µL. *Alternatively, when applying other sample volumes than 200 µL in step 3, fill up with PSB to a final volume of 600 µL (e.g., 100 µL sample + 500 µL PSB).*	**+ 400 µL PSB**
5	**Add Quantification Reagent QR** Add **400 µL Quantification Reagent QR** to each microcentrifuge tube (dilution series and protein samples). **Shake tubes** until a complete color change from blue to yellow occurs. *Caution: Quantification Reagent QR contains hydrochloric acid. Wear protective clothing and goggles.*	**+ 400 µL QR** **Shake tubes**
6	**Incubate** Incubate microcentrifuge tubes for **30 min** at room temperature. Shake tubes after incubation. **Do not centrifuge tubes** at this point. *A variation in incubation time may result in reduced signal and loss of sensitivity. An incubation time of 30 ± 5 min is recommended.*	**Incubate 30 min**
7	**Measure light extinction** Transfer the solution of each tube to a suitable semi-micro cuvette. Measure light extinction photometrically at **570 nm**. *Light extinction can be measured in the range of 530 – 700 nm. Typical correlation coefficients (concentration of BSA versus extinction value) of 0.97 – 1.00 are obtained within this wavelength range.*	**Measure extinction at 570 nm**
8	**Calculate protein concentration** Calculate protein concentration of samples in relation to the BSA dilution series. *Make sure that the protein concentration of your sample lies within the range of the largest (#1) and the smallest (#6) concentration of the calibration curve in order to obtain valid measurements. Do not extrapolate beyond this range.*	**Calculate protein concentration**

5.3 Microcuvette assay procedure

Before starting the preparation:

- Check if the BSA reference protein stock solution was prepared according to section 3.
- Make sure that there are no precipitates in Protein Solving Buffer PSB and in the reverence protein (BSA) solution (if necessary, heat to approx. 30 °C).

1 Prepare a BSA (reference protein) dilution series

Number seven reaction tubes according to column A (see table below; #1: BSA stock solution).

Add **50 µL Protein Solving Buffer PSB** to tubes #2–#7 (column B).

Add **BSA solution** to tubes #2–#6 according to column C.

The resulting protein concentration and amount are shown in columns D and E.

A	B	C	D	E
Tube	**Add PSB to tube**	**Add BSA solution to tube**	**Resulting BSA concentration**	**Resulting BSA in 20 µL**
#1	BSA stock solution		1 µg/µL	20 µg
#2	50 µL	50 µL from tube #1	0.5 µg/µL	10 µg
#3	50 µL	50 µL from tube #2	0.25 µg/µL	5 µg
#4	50 µL	50 µL from tube #3	0.125 µg/µL	2.5 µg
#5	50 µL	50 µL from tube #4	0.063 µg/µL	1.25 µg
#6	50 µL	50 µL from tube #5	0.031 µg/µL	0.625 µg
#7	50 µL	–	0 µg/µL	0 µg

The prepared BSA dilutions series is sufficient for the determination of **one** calibration curve. Freeze BSA stock solution for storage. Keep dilution series on ice during use and dispose all dilutions at the end of a working day.

2 Dispense dilution series into microcentrifuge tubes — **40 µL of dilution series**

Pipette **40 µL** of **each dilution series solution** (#1–#7) into 1.5 mL microcentrifuge tubes (not supplied).

(#1: BSA stock solution; #2–#6: BSA dilutions; #7: BSA-free PSB)

3 Dispense your protein samples — **40 µL of samples**

Pipette **40 µL** of your **samples** to (new) microcentrifuge tubes.

Alternatively, 1 – 120 µL of sample can be applied.

4	**Fill up dilution series and protein samples** Add **80 µL PSB** to each well (dilution series and protein samples). Final volume is 120 µL. *Alternatively, when applying other sample volumes than 40 µL in step 3, fill up with PSB to a final volume of 120 µL (e.g., 10 µL sample + 110 µL PSB).*	**+ 80 µL PSB**
5	**Add Quantification Reagent QR** Pipette **80 µL Quantification Reagent QR** to each tube (dilution series and protein samples). **Shake tube** until a complete color change from blue to yellow occurs. *Caution: Quantification Reagent QR contains hydrochloric acid. Wear protective clothing and goggles.*	**+ 80 µL QR** **Shake tube**
6	**Incubate** Incubate tubes for **30 min** at room temperature. Shake tubes after incubation. **Do not centrifuge** tubes at this point! A variation in incubation time may result in reduced signal and loss of sensitivity. An incubation time of 30 ± 5 min is recommended.	**Incubate 30 min**
7	**Measure light extinction** Transfer the solution of each tube to a suitable microcuvette. Measure light extinction photometrically at **570 nm**. *Light extinction can be measured in the range of 530 – 700 nm. Typical correlation coefficients (concentration of BSA versus extinction value) of 0.97 – 1.00 are obtained within this wavelength range.*	**Measure extinction at 570 nm**
8	**Calculate protein concentration** Calculate protein concentration of samples in relation to the BSA dilution series. *Make sure that the protein concentration of your sample lies within the range of the largest (#1) and the smallest (#6) concentration of the calibration curve in order to obtain valid measurements. Do not extrapolate beyond this range.*	**Calculate protein concentration**

5.4 Low volume assay procedure

Before starting the preparation:

- Check if the BSA reference protein stock solution was prepared according to section 3.
- Make sure that there are no precipitates in Protein Solving Buffer PSB and in the reverence protein (BSA) solution (if necessary, heat to approx. 30 °C).

1 Prepare a BSA (reference protein) dilution series

Number six reaction tubes according to column A (see table below; #1: BSA stock solution).

Add **20 µL Protein Solving Buffer PSB** to tubes #2–#6 (column B).

Add **BSA solution** to tubes #2–#5 according to column C.

The resulting protein concentration and amount are shown in columns D and E.

A Tube	B Add PSB to tube	C Add BSA solution to tube	D Resulting BSA concentration	E Resulting BSA in 20 µL
#1	BSA stock solution		1 µg/µL	7.5 µg
#2	20 µL	20 µL from tube #1	0.5 µg/µL	3.75 µg
#3	20 µL	20 µL from tube #2	0.25 µg/µL	1.88 µg
#4	20 µL	20 µL from tube #3	0.125 µg/µL	0.94 µg
#5	20 µL	20 µL from tube #4	0.063 µg/µL	0.47 µg
#6	20 µL	–	0 µg/µL	0 µg

The prepared BSA dilutions series is sufficient for the determination of **two** calibration curves. Freeze BSA stock solution for storage. Keep dilution series on ice during use and dispose all dilutions at the end of a working day.

2 **Dispense dilution series into microcentrifuge tubes** — **7.5 µL of dilution series**

Pipette **7.5 µL** of **each dilution series solution** (#1–#6) into 1.5 mL microcentrifuge tubes (not supplied).

(#1: BSA stock solution; #2–#5: BSA dilutions; #6: BSA-free PSB)

3 **Dispense your protein samples** — **7.5 µL of samples**

Pipette **7.5 µL** of your **samples** to (new) microcentrifuge tubes.

4 **Fill up dilution series and protein samples**

Not necessary! Proceed directly with step 5.

5	**Add Quantification Reagent QR** Add **5 µL Quantification Reagent QR** to each tube (dilution series and protein samples). **Mix (e.g., by pipetting up and down)** until a complete color change from blue to yellow occurs. Caution: Quantification Reagent QR contains hydrochloric acid. Wear protective clothing and goggles.	**+ 5 µL QR** **Mix**
6	**Incubate** Incubate tubes for **30 min** at room temperature. Shake tubes after incubation. **Do not centrifuge** at this point! *A variation in incubation time may result in reduced signal and loss of sensitivity. An incubation time of 30 ± 5 min is recommended.*	**Incubate 30 min**
7	**Measure light extinction** Transfer 10 µL of the solution of each tube to a suitable **low volume photometer with 1 mm path length**. Measure light extinction photometrically at **570 nm**. Avoid bubbles in the solution because they severely disturb the measurement. *Caution: The solution to be measured contains HCl; check the compatibility of your instrument with HCl. Do not spill. Immediately remove solution from the photometer after measurement.* *Light extinction can be measured in the range of 530 – 700 nm. Typical correlation coefficients (concentration of BSA versus extinction value) of 0.97 – 1.00 are obtained within this wavelength range.*	**Measure extinction at 570 nm**
8	**Calculate protein concentration** Calculate protein concentration of samples in relation to the BSA dilution series. *Make sure that the protein concentration of your sample lies within the range of the largest (#1) and the smallest (#5) concentration of the calibration curve in order to obtain valid measurements. Do not extrapolate beyond this range.*	**Calculate protein concentration**

6 Appendix

6.1 Guidance for data analysis – calculation of protein concentration

For calculation of protein concentration of unknown samples it is necessary to prepare a BSA (reference protein) dilution series that is generated from known protein concentrations.

As guidance for calculation please follow each calculation steps listed below as an example.

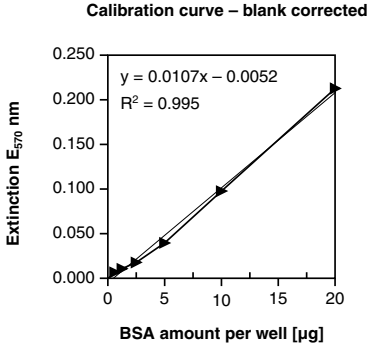
1) Measure the extinction of the reference protein dilution series and your unknown samples (US).

#	BSA amount per well [µg]	Absorption of reference protein dilution series	US **A**	US **B**	US **C**	US **D**	US **E**	US **F**	US **G**
1	20	0.245	0.040	0.211	0.100	0.045	0.345	0.033	0.111
2	10	0.130	0.042	0.166	0.088	0.040	0.354	0.031	0.132
3	5	0.072	0.037	0.199	0.111	0.046	0.330	0.032	0.250
4	2.5	0.050							
5	1.25	0.043							
6	0.625	0.039							
7 blank	0	**0.032**							
	Correlation coefficient	0.998							

2) The correct raw data is obtained by subtracting the blank value from the values of the protein standards and unknown samples.

#	BSA amount per well [µg]	Reference protein dilution series	US **A**	US **B**	US **C**	US **D**	US **E**	US **F**	US **G**
1	20	0.213	0.008	0.179	0.068	0.013	0.313	0.001	0.079
2	10	0.098	0.011	0.134	0.056	0.008	0.322	-0.002	0.100
3	5	0.040	0.005	0.167	0.079	0.014	0.298	-0.001	0.218
4	2.5	0.018							
5	1.25	0.011							
6	0.625	0.007							
7 blank	0	0							

3) Create a standard curve by plotting the extinction values versus the reference protein amount per well. Plot a linear regression for the set of standards and calculate the equation of this line.



In this case the equation is y = 0.0107x - 0.0052.

4) Calculate protein concentration.

Insert the measured extinction of each unknown sample for x (amount of protein per well) to calculate the protein amount of your unknown sample

$y = a\,x + b$
$x = (y - b) / a$

a = 0.0107 (slope)
b = 0.0052 (axis intercept)
y = extinction value (blank corrected)
x = protein amount in well [µg]

Calculation example:

Value from unknown sample A = 0.008
$0.008 = 0.0107\,x - 0.0052$
$x = (0.008 + 0.0052) / 0.0107$
x = 1.2 µg

			Calculated protein amount per well [µg]						
#	**BSA amount per well [µg] nominal**	**Reference protein dilution series [µg] measured and calculated**	US **A**	US **B**	US **C**	US **D**	US **E**	US **F**	US **G**
1	20	20	1.2	17	7	1.7	30	0.5	8
2	10	9	1.5	13	6	1.2	31	0.4	10
3	5	4	0.9	16	8	1.8	28	0.4	21
4	2.5	2				**Mean value**			
5	1.25	0.9	**1.2**	**15**	**7**	**1.6**	**30**	**0.4**	**13**
6	0.625	0.5							
7 blank	0	0							

If 20 µL from each protein sample was pipetted into each well, the protein concentration within this 20 µL sample is calculated by:

Mean value/20 µL = protein concentration [µg/µL]

US **A**	US **B**	US **C**	US **D**	US **E**	US **F**	US **G**
Mean value protein amount [µg]						
1.2	15	7	1.6	30	0.4	13

Protein concentration [µg/µL]						
0.06	0.75	0.35	0.08	1.5	0.02	0.65

5) Interpretate the results.

- Results within the range of the reference dilution series are trustworthy.
- Results higher than for the most concentrated reference dilution should be considered with care. Do not extrapolate, just interpolate. Remeasure your sample with a smaller aliquot.
- Results smaller than for the most diluted reference protein sample should be interpreted with care. Remeasure the sample using a larger aliquot.

6.2 Troubleshooting

Problem	Possible cause and suggestions
Lowest value of calibration curve cannot be measured	*Storage of dilution series* • Do not store dilution series of the BSA reference protein. Prepare fresh dilution series. • Freeze the BSA stock solution for storage. *Photometer, microplates or cuvettes* • Sensitivity of the assay may be influenced by the type of photometer, microplates, or cuvettes used. If the lowest calibration point is not discriminated against background, prepare a calibration series with higher BSA amounts.
Samples appear turbid after addition of Quantification Reagent QR	*High protein concentration* • As long as the measured extinction of your sample falls within the range of the calibration curve, this is acceptable.
Varying results upon multiple measurements	*Samples not mixed immediately before extinction measurement* • Shake microplate immediately before extinction measurement. • Shake reaction tubes after incubation and before transfer to semi-micro cuvettes. After transfer of samples to semi-micro cuvettes, measure extinction immediately. • Strictly keep to the recommended incubation time. • Do not centrifuge at any time after addition of Quantification Reagent QR. • Avoid bubble formation and foaming, especially for protocol section 5.1. (microplate assay procedure). Light scattering caused by foam has impact on turbidity measrurements.

Problem	Possible cause and suggestions
Protein Solving Buffer PSB appears turbid	*Low storage temperature* • Warm PSB to approx. 30 °C.
Similar extinction for all dilution series samples	*Fill-level of semi-micro or microcuvette not compatible with photometer* • Make sure that the sample volume in the semi-micro cuvette is high enough to let the light beam pass through the solution. Consult your photometer user manual. Check the compatibility of disposable cuvettes used with your photometer – consider light beam center height and cuvette fill volume.

6.3 Ordering information

Product	REF	Number of assays or preparations
Protein Quantification Assay	740967.50/.250	50/250
NucleoSpin® RNA/Protein	740933.10/50/.250	10/50/250
NucleoSpin® TriPrep*	740966.10/50/.250	10/50/250
Porablot transfer membranes	see ***www.mn-net.com/bioanalysis***	
Blotting paper	see ***www.mn-net.com/bioanalysis***	

6.4 References

Bradford MM (1976): A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248 – 254.

Karlsson JO *et al.* (1994): A method for protein assay in Laemmli buffer. Analytical Biochemistry 219, 144 – 146.

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6.5 Product use restriction / warranty

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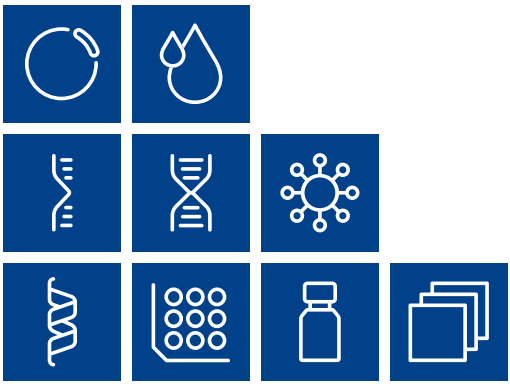
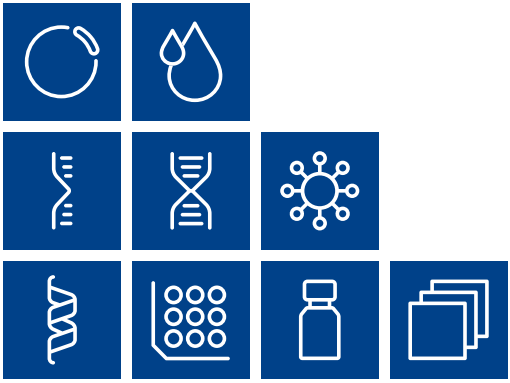


Plasmid DNA
Clean up
RNA
DNA
Viral RNA and DNA
Protein
High throughput
Accessories
Auxiliary tools



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