INSTRUCTION MANUAL

SERVA Purple Protein Quantification Assay

Kit for protein quantification

(/Cat. No. 39235)

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Content

1. SERVA Purple Protein Quantification Assay

1.1. General Information

SERVA Purple Protein Quantification Assay offers a complete protein quantification assay. The assay is significantly more sensitive than existing standard colorimetric measurements (ninhydrin, Lowry, BCA). It is an eco-friendly fluorescent dye that covalently and reversibly binds to lysine, arginine and histidine residues in proteins and peptides to yield an intensely red-fluorescent product.

This unique mechanism allows highly sensitive quantification of proteins and peptides over a wide linear dynamic range. Fluorescence intensity is directly proportional to protein concentration; consequently, big differences in protein concentrations generate commensurately big differences in fluorescence intensity.

Moreover, SERVA Purple Protein Quantification Assay generates intuitive results and exhibits enhanced robustness to instrument variability.

This assay exhibits very low protein-to-protein variation, leading to more accurate protein concentration values. It can effectively stain glycosylated proteins, phospho-proteins, crosslinked (disulfide containing) proteins, metalloproteins, hydrophobic proteins, and lipoproteins.

Benefits:

- Disposable, non-toxic dye
- No requirement of potassium cyanide
- No requirement of any heat or reduction step
- Accurate quantification of peptides and glycol-, phospho-, non-reduced and lipoproteins
- No denaturing or precipitation of the sample, which allows subsequent assays
- Compatible with 1D / 2D, MS and HPLC

1.2. Components

Component	2,000 Assays
SERVA Purple Protein Quantification Assay	10 l

1.3. Additionally required equipment

- High-purity water, e.g. distilled, Milli Q or equivalent
- Bicarbonate buffer (0.1 M Sodium bicarbonate-sodium carbonate buffer, pH 9)
- 1.5 ml-Microcentrifuges tubes
- Pipettes
- Black 384 or 96 well plates; alternatively, fluorimeter cuvettes
- Fluorescence plate reader capable of reading at an excitation wavelength of 518 nm and an emission wavelength of 605 nm

1.4. Storage conditions

Upon receipt, store the dye light protected at + 2°C to + 8 °C (up to 6 months) in the original brown bottle provided.

For long term storage, store the dye at -15°C to - 30°C.

1.5. Excitation and emission spectra

The excitation and emission maxima for the SERVA Purple dye (when bound to protein) are ~518 nm and ~610 nm, respectively. To take full advantage of the fluorescence signal, it is ideal to choose filters that are spectrally separated and slightly offset from the peak excitation and emission wavelengths.
The excitation and emission spectra of SERA Purple can be seen in Figure 1.

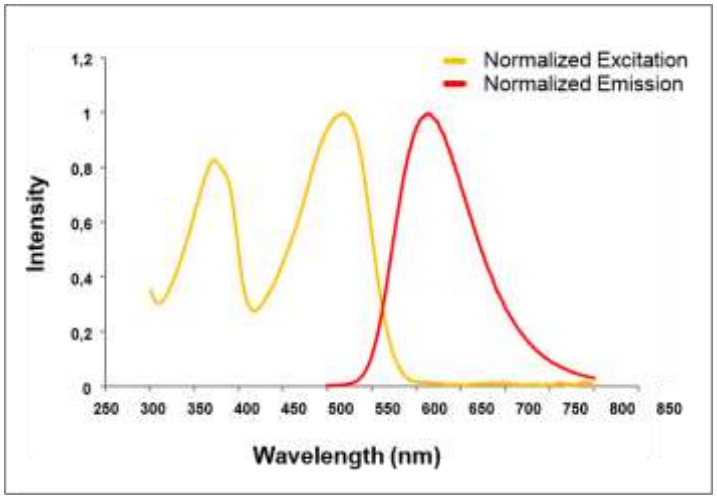

Fig. 1: Excitation and Emission Spectra of SERVA Purple dye with BSA.

2. Procedure of the SERVA Purple Protein Quantification Assay

Assay volumes can range from 20 µl in 384 well plates to 200 µl in 96 well plates. The kit works equally well for larger volumes (3 ml in cuvette). Larger volumes have been shown to increase the upper limit of protein quantification. We recommend using 96 well plates with a final volume of 100 µl.

The kit is compatible with most industry-standard fluorescent imaging and recording systems that can excite with ultraviolet, blue, or green light, and record red light emission. This includes multiwall plate-based fluorimeters. Laser-based imaging systems are also highly suitable.

Note: The detection limits are largely determined by the sensitivity limits of the fluorescence instrumentation employed.

2.1. Preparation of buffers, standards and reagents

Before assays, prepare Bicarbonate Buffer, Peptide Standard Solution and Working Reagent as described below.

Except for Bicarbonate Buffer, solutions should be freshly prepared.

2.1.1. Bicarbonate Buffer

Prepare a 0.1 M solution of Sodium Bicarbonate-Sodium Carbonate Buffer.
The pH of the Bicarbonate Buffer should be approximately 9.

10 ml 100 mM Na_2CO_3 + 115 ml100 mM $NaHCO_3$, add ddH_2O to a final volume of 500 ml. The pH of this buffer is approx. 9.0 - 9.2.

2.1.2. Standard Solution

To obtain a standard curve for calculation of the protein/peptide concentration in the sample it is necessary to use standards.

Prepare serial dilutions of a protein or peptide in water or sample buffer (e.g. a 4-fold serial dilution ranging from 0.655 mg/ml to 40 ng/ml is recommended, see Table 1 for preparing a 4-fold serial dilution).

The standard solution should be prepared using BSA or the same species and buffer as the sample to be quantified.

Tube no.	**Water or sample buffer**	**Protein/Peptide or BSA solution**	**Final protein concentration of the standard solution**
1	-	1 volume (655,360 ng/mL)	655,360 ng/mL
2	3 volumes	1 volume (655,360 ng/mL)	163,840 ng/mL
3	3 volumes	1 volume (163,840 ng/mL)	40,960 ng/mL
4	3 volumes	1 volume (40,960 ng/mL)	10,240 ng/mL
5	3 volumes	1 volume (10,240 ng/mL)	2,560 ng/mL
6	3 volumes	1 volume (2,560 ng/mL)	640 ng/mL
7	3 volumes	1 volume (640 ng/mL)	160 ng/mL
8	3 volumes	1 volume (160 ng/mL)	40 ng/mL

Tab. 1: Preparation of a 4-fold serial dilution for protein standard curve

2.1.3. Working Solution

If the SERVA Purple dye has been stored at - 20 °C, allow warming to room temperature. Mix gently before use. Prepare a working solution of dye by mixing it with the bicarbonate buffer in a ratio of 1:9 (see Tab. 2 for appropriate dilutions).

Number of assays			Volume to prepare		
1 mL cuvette assay	**100 µl assays (96-well plate)**	**20 µl assays (384-well plate)**	**Dye [µL]**	**Bicarbonate Buffer [µL]**	**Total volume of working solution [µL]**
1	**10**	50	50	450	**500**
5	**50**	250	250	2,250	**2,500**
10	**100**	500	500	4,500	**5,000**
50	**500**	2,500	2,500	22,500	**25,000**
100	**1,000**	5,000	5,000	45,000	**50,000**
200	**2,000**	10,000	10,000	90,000	**100,000**

Tab. 2: Volumes of SERVA Purple dye working solution to prepare

2.2. Assay Protocol

Step	Notes/Protocol
Blank	Prepare the blank by mixing equal volumes of working solution and water/buffer.
Protein Standard	Add equal volumes of working solution to each standard solution.
Sample	Add an equal volume of working solution to a sample of unknown concentration.
Assay	**Note:** Larger or smaller volumes may be used if desired. **For a 1 mL cuvette assay:** Add 500 µL of sample / buffer / standard to 500 µL of working solution. **For a microtiter (96-well) plate (100 µL) assay:** Add 50 µL of sample / buffer / standard to 50 µL of working solution. **For a 384-well plate (20 µL) assay:** Add 10 µL of sample / buffer / standard to 10 µL of working solution.
Incubation	30 min light protected at room temperature
Fluorescence measurement	Measure fluorescence: λ_{exc}: 518 nm, λ_{em}: 605 nm Subtract background fluorescence of the blank from all other values. Under these conditions, the signal is stable up to 6 hours. If longer storage periods are required, it is recommended to seal the plates and store at + 2 to + 8 °C.
Standard curve	**Note:** A linear fit is usually used, but a larger dynamic range can be achieved with an exponential fit. Create a standard curve by plotting fluorescence over protein standard concentrations (log10 fluorescence vs log10 protein concentration).
Results	Use the standard curve to determine the protein concentration of the unknown sample.

3. Important to know

- Use high-grade chemicals and freshly prepare any reagents that are unstable.
- Use microtiter plates that are suited for fluorescence measurements.
- Prepare working solution fresh for each assay.
- Ideally, the same buffer should be used for the protein standard and the sample of unknown concentration.

- SERVA Purple dye reacts with primary amines.
  Therefore, these should be avoided in samples and buffers.
- The assay is suitable for quantification of most proteins and peptides but individual standard curves are required for each peptide.

4. Interfering Compounds

Interfering compounds (see Tab. 3) should be at or below the given concentrations.

IMPORTANT:
Tab. 3 is not a complete list of incompatible compounds.

The protein of interest may be assayed in ultrapure water alone instead of sample buffer with possible interfering substances.
Comparison of the readings will indicate if interference exists.
Alternatively, the interfering substance may be removed using dialysis or protein precipitation.

Substance	Max. concentration
2-Mercaptoethanol	20 mM
Acetonitrile (ACN)	0.5 %
$CaCl_2$	500 µM
CHAPS	0.05 %
Dithiothreitol (DTT)	1.5 mM
EDTA	20 mM
Formic acid	0.01 %
Glycerol	25 %
HCl	500 µM
Iodoacteamide	50 mM
NaCl	100 mM
$(NH_4)_2CO_3$	500 µM
NP40	0.005%
Sodiumdodecylsulfate (SDS)	0.1 %
Sucrose	250 mM
Tributylphosphate (TBP)	10 mM
Tris-(2-carboxyethyl)-phosphine hydrochloride (TCEP)	2 mM
Trifluoroacetic acid (TFA)	0.005 %
Thiourea	500 mM
Tris	500 µM
Triton® X-100	0.005 %
Tween®	0.01 %
Urea	1 M

Tab.3: Interfering compounds