

2-D Quant Kit

Product Specification Sheet

The 2-D Quant Kit is designed for the accurate determination of protein concentration in samples to be analyzed by high resolution electrophoresis techniques such as 2-D electrophoresis, SDS-PAGE or IEF. Many of the reagents used in the preparation of such samples, including detergents, reductants, chaotropes and carrier ampholytes, are incompatible with other protein assays. The procedure works by quantitatively precipitating proteins while leaving interfering substances in solution. The assay is based on the specific binding of copper ions to protein. Precipitated proteins are resuspended in a copper-containing solution and unbound copper is measured with a colorimetric agent. The color density is inversely related to the protein concentration. The assay has a linear response to protein in the range of 0–50 µg. The procedure is compatible with such common sample preparation reagents as 2% SDS, 1% DTT, 8 M urea, 2 M thiourea, 4% CHAPS, 2% Pharmalyte™ and 2% IPG Buffer. The kit contains reagents sufficient for 500 data points.

Warning

For research use only.
Not recommended or intended for diagnosis of disease in humans or animals.
Do not use internally or externally in humans or animals.

Storage

The kit should be stored at 4–8°C

Function testing

Each lot of the 2-D Quant Kit is tested for its ability to accurately quantify a standard protein solution.

Safety warnings and precautions

All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.

Components

Precipitant:

This solution renders proteins insoluble.

Co-precipitant:

This solution contains reagents that co-precipitate with proteins and enhances their removal from solution

Copper solution:

Precipitated protein is resuspended with this solution.

Color reagent A:

This solution is mixed with color reagent B to prepare the color reagent used to measure unbound copper ion.

Color reagent B:

This solution is mixed with color reagent A to prepare the color reagent used to measure unbound copper ion.

Bovine serum albumin standard solution:

This solution is used to prepare a standard curve.

Overview

Electrophoretic analysis of protein samples requires accurate quantification of the sample to be analyzed. This assures that an appropriate amount of protein is loaded. In addition, accurate quantification facilitates comparison among similar samples by allowing identical amounts of protein to be loaded. Accurate quantification of samples prepared for electrophoresis is, however, difficult because many of the reagents used to prepare and solubilize samples for electrophoresis are incompatible with common protein assays.

Current spectrophotometric methods of quantifying protein rely either on Coomassie™ dye binding (1) or protein-catalyzed reduction of cupric (Cu²⁺) ion to cuprous (Cu⁺) ion (2–4). Dye-binding assays cannot be used in the presence of any reagent that also binds Coomassie dye. This includes carrier ampholytes such as Pharmalyte and IPG Buffer and detergents such as CHAPS, SDS or Triton™ X-100. Assays that depend on the reduction of cupric ion cannot be used in the presence of reductants such as DTT, or in the presence of reagents that form complexes with cupric ion, such as thiourea or EDTA.

Samples prepared for electrophoresis (including SDS-PAGE and IEF) are often difficult to quantify due to the presence of detergent and reductant. 2-D electrophoresis samples represent a particularly difficult quantification challenge due to the possible presence of interfering carrier ampholyte and thiourea in addition to the detergents and reductants typically used in sample preparation for electrophoresis.

The 2-D Quant Kit circumvents these limitations and can be used to accurately quantify protein samples prepared for 2-D electrophoresis as well as any sample that contains substances that otherwise interfere with protein quantification. The procedure uses a combination of a unique precipitant and co-precipitant to quantitatively precipitate sample protein while leaving interfering contaminants in solution. The protein is pelleted by centrifugation and resuspended in an alkaline solution of cupric ions. The cupric ions bind to the polypeptide backbones of any protein present. A colorimetric agent which reacts with unbound cupric ions is then added. The color density is inversely related to the concentration of protein in the sample. Protein concentration can be accurately estimated by comparison to a standard curve. Since the assay does not depend on reaction with protein side groups, reactivity is largely independent of amino acid composition. There is little protein to protein variation in the response of this assay.

Protein samples can be quantified with the 2-D Quant Kit directly in electrophoresis sample solution. This simplifies sample preparation and assures that the protein assay result accurately reflects the protein concentration of the sample.

The volume range of the assay is 1–50 µl and the linear range for quantification is 0–50 µg. This corresponds well with typical volumes and concentrations of samples prepared for 2-D electrophoresis.

This product is covered by US patent US 5900367.



Protocol

Introduction

The assay volume is 1.5 ml, which is compatible with most spectrophotometer cuvettes. However, larger or smaller assay volumes may be accommodated by proportionally scaling the volumes of the sample, standard curve and assay reagents.

The accuracy of this assay is largely determined by the technique of the user. Careful pipetting of standard curve and sample solutions is essential. If the sample is viscous, it may be difficult to accurately pipette volumes below 10 μ l. More accurate results may be obtained by diluting a concentrated sample into water prior to assaying. Performing the assay in duplicate is recommended.

Unlike most protein assays, the absorbance of the assay solution decreases with increasing protein concentration. Do not subtract the blank reading from the sample reading.

The volume range of the assay is 1–50 μ l and the linear range for quantification is 0–50 μ g. The assay has a sensitivity threshold of 0.5 μ g.

Hint: Always position the microcentrifuge tubes in the centrifuge rotor with the cap hinge facing outward. This way the pellet will always be on the same side of the tube so it can be left undisturbed, minimizing loss.

The assay should be performed at room temperature.

Required but not provided:

- 2 ml microcentrifuge tubes
- Vortex mixer
- Microcentrifuge
- Visible light spectrophotometer (e.g. Novaspec II or Ultrospec 1000pro)

Preliminary preparations

Prior to performing the assay, prepare an appropriate volume of working color reagent by mixing 100 parts of color reagent A with 1 part color reagent B. Each individual assay requires 1 ml of working color reagent.

Working color reagent can be stored at 4–8°C for up to one week or as long as the optical absorbance (A_{480}) of the solution remains below 0.025 at 480 nm.

Standard procedure

1. Prepare a standard curve according to Table 1 using the 2 mg/ml Bovine serum albumin (BSA) standard solution provided with the kit. Set up six tubes and add standard solution according to Table 1. Tube 1 is the assay blank, which contains no protein.

Table 1: Preparation of standard curve

Tube number	1	2	3	4	5	6
Volume of 2 mg/ml						
BSA standard solution	0 μ l	5 μ l	10 μ l	15 μ l	20 μ l	25 μ l
Protein quantity	0 μ g	10 μ g	20 μ g	30 μ g	40 μ g	50 μ g

Note: The accuracy of the assay is unaffected by the volume of the sample as long as the sample volume is 50 μ l or less. It is therefore unnecessary to dilute standard or sample solutions to a constant volume.

2. Prepare tubes containing 1–50 μ l of the sample to be assayed. Duplicates are recommended. The useful range of the assay is 0.5–50 μ g and it is also recommended that more than one sample volume or dilution be assayed for each sample to ensure that the assay falls within this range.
3. Add 500 μ l precipitant to each tube (including the standard curve tubes). Vortex briefly and incubate the tubes 2–3 min at room temperature.
4. Add 500 μ l co-precipitant to each tube and mix briefly by vortexing or inversion.
5. Centrifuge the tubes at a minimum of 10 000 \times g for 5 min. This sediments the protein.
6. Remove the tubes from the centrifuge as soon as centrifugation is complete. A small pellet should be visible. Decant the

supernatants. Proceed rapidly to the next step to avoid resuspension or dispersion of the pellets.

7. Carefully reposition the tubes in the microcentrifuge as before, with the cap-hinge and pellet facing outward. Centrifuge the tubes again to bring any remaining liquid to the bottom of the tube. A brief pulse is sufficient. Use a micropipette to remove the remaining supernatant. There should be no visible liquid remaining in the tubes.
 8. Add 100 μ l of copper solution and 400 μ l of distilled or de-ionized water to each tube. Vortex briefly to dissolve the precipitated protein.
 9. Add 1 ml of working color reagent to each tube (See "Preliminary preparations" for preparing the working color reagent). Ensure instantaneous mixing by introducing the reagent as rapidly as possible. Mix by inversion.
 10. Incubate at room temperature for 15–20 min.
 11. Read the absorbance of each sample and standard at 480 nm using water as the reference. The absorbance should be read within 40 min of the addition of working color reagent (step 9).
- Note:** Unlike most protein assays, the absorbance of the assay solution decreases with increasing protein concentration. Do not subtract the blank reading from the sample reading or use the assay blank as the reference.
12. Generate a standard curve by plotting the absorbance of the standards against the quantity of protein. Use this standard curve to determine the protein concentration of the samples.

Procedure for Ettan™ DIGE

We recommend the following procedure for 2-D Fluorescence Difference Gel Electrophoresis (2-D DIGE).

1. Prepare a standard curve according to Table 2 using the 2 mg/ml Bovine serum albumin (BSA) standard solution provided with the kit. Set up six tubes and add standard solution according to Table 1. Tube 1 is the assay blank, which contains no protein.

Table 2: Preparation of standard curve

Tube number	1	2	3	4	5	6
Volume of 2 mg/ml						
BSA standard solution	0 μ l	5 μ l	10 μ l	15 μ l	20 μ l	25 μ l
Protein quantity	0 μ g	10 μ g	20 μ g	30 μ g	40 μ g	50 μ g

To each standard (including the 0 μ g) add x μ l of the sample buffer to bring the total to the same as the sample.

Note: The accuracy of the assay is unaffected by the volume of the sample as long as the sample volume is 50 μ l or less. It is therefore unnecessary to dilute standard or sample solutions to a constant volume.

2. Prepare tubes containing 1–50 μ l of the sample to be assayed. Duplicates are recommended. The useful range of the assay is 0.5–50 μ g and it is also recommended that more than one sample dilution be assayed for each sample to ensure that the assay falls within this range. When preparing dilutions it is important to dilute the sample in the same sample buffer used as for the original test sample to keep the amount of sample buffer added to the standard curve consistent.
3. Add 500 μ l precipitant to each tube (including the standard curve tubes). Vortex briefly and incubate the tubes 2–3 min at room temperature.
4. Add 500 μ l co-precipitant to each tube and mix briefly by vortexing or inversion.
5. Centrifuge the tubes at a minimum of 10 000 \times g for 5 min. This sediments the protein.
6. Remove the tubes from the centrifuge as soon as centrifugation is complete. A small pellet should be visible. Decant the supernatants.
7. Repeat steps 3 to 6. Proceed rapidly to the next step to avoid resuspension or dispersion of the pellets.

8. Carefully reposition the tubes in the microcentrifuge as before, with the cap-hinge and pellet facing outward. Centrifuge the tubes again to bring any remaining liquid to the bottom of the tube. A brief pulse is sufficient. Use a micropipette to remove the remaining supernatant. There should be no visible liquid remaining in the tubes.
9. Add 100 μ l of copper solution and 400 μ l of distilled or de-ionized water to each tube. Vortex briefly to dissolve the precipitated protein.
10. Add 1 ml of working color reagent to each tube (See "Preliminary preparations" for preparing the working color reagent). Ensure instantaneous mixing by introducing the reagent as rapidly as possible. Mix by inversion.
11. Incubate at room temperature for 15–20 min.
12. Read the absorbance of each sample and standard at 480 nm using water as the reference. The absorbance should be read within 40 min of the addition of working color reagent (step 9).

Note: Unlike most protein assays, the absorbance of the assay solution decreases with increasing protein concentration. Do not subtract the blank reading from the sample reading or use the assay blank as the reference.

13. Generate a standard curve by plotting the absorbance of the standards against the quantity of protein. Use this standard curve to determine the protein concentration of the samples.

Tolerance to commonly used reagents

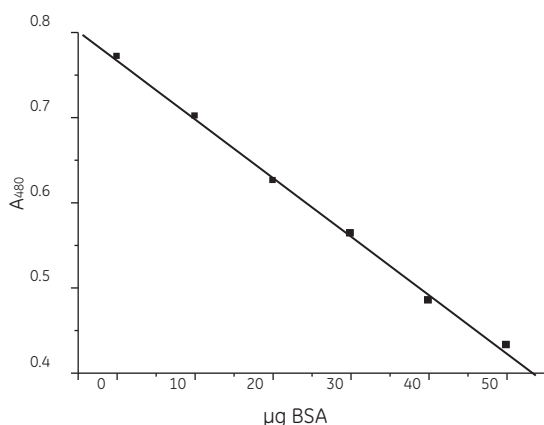
The 2-D Quant Kit can be used to accurately quantify protein in the presence of the following reagents, either individually or in combination.

Table 3: Compounds tested for assay compatibility

compound tested	concentration
SDS	2% (w/v)
CHAPS	4% (w/v)
Triton X-100	1% (w/v)
Pharmalyte pH 3-10	2% (v/v)
IPG Buffer pH 3-10 NL	2% (v/v)
Tris™	50 mM
EDTA	10 mM
DTT	1% (65 mM)
2-Mercaptoethanol	2% (v/v) Urea 8 M
Urea	8 M
Thiourea	2 M
Glycerol	30% (w/v)

Standard curve example

Fig 1. Typical standard curve



References

1. Bradford, M.M., *Anal. Biochem.* **72**, 248 (1976).
2. Goshev, I., and Nedkov, P. *Anal. Biochem.* **95**, 340 (1979).
3. Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J., *J. Biol Chem.* **193**, 265 (1951).
4. Smith, P.K., Krohn, R.I., Hermanson, G.T., Mallia, A.K., Gartner, F.H., Provenzano, M.D., Fujimoto, E.K., Goeke, N.M., Olson, B.J., and Klenk, D.C., *Anal. Biochem.* **150**, 76 (1985).

Troubleshooting guide

symptom: The relationship between protein concentration and absorbance at 480 nm is not linear (the standard curve is not straight).

possible cause	remedy
Insufficient centrifugation (Step 5).	Ensure that the protein is fully sedimented and forms a tight pellet by concentration centrifuging for at least 5 min at a minimum of 10 000 × g
The working color reagent was not the sample (Step 9).	Ensure instantaneous mixing by introducing the working color reagent to the mixed rapidly with tube as rapidly as possible. Mix by inversion or vortexing as soon as possible after introducing the reagent.

symptom: Evidence of interference (absorbance at 480 nm is anomalously low regardless of the protein concentration in the sample).

possible cause	remedy
Carry over of interfering reagents in the protein solution.	Ensure that the supernatant is completely removed following centrifugation (Step 7). There should be no visible liquid remaining in the tubes. If there is a high level of interfering contaminants in the sample solution, it may be necessary to wash the precipitate as follows between Steps 7 and 8. After removing the supernatant, add 500 μ l of precipitant to each tube. Add 100 μ l of co-precipitant. Cap the tube and gently invert a few times without disturbing the pellet. Repeat Steps 5 through 7 and continue.

symptom: Protein concentration determined is inconsistent or anomalously low (absorbance at 480 nm is anomalously high).

possible cause	remedy
The protein was not fully resuspended following centrifugation (Step 8).	Some sample solution components may result in a protein pellet that is more difficult to resuspend. Vortex the tube (Step 8) for at least 10 s.

Ordering information

Product	Quantity	Code No.
2-D Quant Kit	500 assays	80-6483-56
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Related products	Quantity	Code No.
Tris	500 g	17-1321-01
Urea	500 g	17-1319-01
CHAPS	1 g	17-1314-01
Triton X-100	500 ml	17-1315-01
Dithiothreitol (DTT)	1 g	17-1318-01
Bromophenol Blue	10 g	17-1329-01
Sample Grinding Kit	50 samples, up to 100 mg tissue or cell sample	80-6483-37
2-D Clean-Up Kit	50 samples, 1-100 µl	80-6484-51
SDS-PAGE Clean-Up Kit	50 samples, 1-100 µl	80-6484-70
Mini Dialysis Kit	1 kDa cut-off, up to 250 µl	80-6483-75
Mini Dialysis Kit	1 kDa cut-off, up to 2 ml	80-6483-94
Mini Dialysis Kit	8 kDa cut-off, up to 250 µl	80-6484-13
Mini Dialysis Kit	8 kDa cut-off, up to 2 ml	80-6484-32
2-D Protein Extraction Buffer Trial Kit	for 6 × 10 ml	28-9435-22
2-D Protein Extraction Buffer-I	for 50 ml	28-9435-23
2-D Protein Extraction Buffer-II	for 50 ml	28-9435-24
2-D Protein Extraction Buffer-III	for 50 ml	28-9435-25
2-D Protein Extraction Buffer-IV	for 50 ml	28-9435-26
2-D Protein Extraction Buffer-V	for 50 ml	28-9435-27
2-D Protein Extraction Buffer-VI	for 50 ml	28-9435-28
Nuclease Mix	0.5 ml	80-6501-42
Protease Inhibitor Mix	1 ml	80-6501-23

Other reagents

*Pharmalyte

*IPG Buffer

*Vivaspin Sample Concentrators

*See GE Healthcare Catalogue for full selection

Related literature

	Code No.
Handbook: 2-D Electrophoresis Using Immobilized pH Gradients, Principles & Methods.	80-6429-60

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