

## Product information

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# Non-Interfering Protein Concentration Determination Kit

**Catalog #:** SK3071  
**Size:** 250 preps  
**Storage:** Mixed components storage\*

\*: Product will be shipped at ambient temperature. Check storage conditions.

### Product Description:

This kit provides a highly sensitive, colorimetric protein assay that overcomes interference of common laboratory agents present in protein solutions, such as detergents (non-ionic, ionic and zwitterionic), reducing agents ( $\beta$ -mercaptoethanol, DTT), chelating agents (EDTA), amines (Tris), sugars, and strong chaotropic buffers. The kit is suitable for protein concentration determination during protein purification, electrophoresis, and many other protein biology applications. A variety of protein samples can be used from cellular fractions, tissue, 2D lysates to chromatography fractions. In this assay, Precipitation Reagent is added to the protein solution to rapidly precipitate total protein. Interfering agents in the supernatant are discarded. Protein concentration is then determined by mixing the protein with an alkaline solution containing a known concentration of copper salt. The copper ions bind to the peptide backbone and the unbound copper is measured with a color-producing agent. As the concentration of protein increases, the concentration of unbound copper decreases, thus the color density is inversely proportional to the amount of protein present in solution. This assay is not affected by protein side chains or groups, which minimizes protein-to-protein variation. This fact, when combined with the strategy of removing interfering agents from the protein solution, allows for a highly reliable determination of protein concentration.

### Features:

- Simple and rapid. The procedure only takes 30 minutes.
- Sensitive. Only 1-50 $\mu$ l of protein sample is required.
- The assay is not affected by the presence of common laboratory agents, such as reducing agents, chelating agents, detergents, amines, sugars, chaotropes, salts, drugs, antibiotics, cobalt and others.
- Linear range: 0.5 $\mu$ g-50 $\mu$ g protein. The kit components are suitable for 250 assays.

### Composition:

Precipitation Reagent I	125 ml
Precipitation Reagent II	125 ml
Copper Solution (Agent I)	25 ml
Color Agent A	250 ml
Color Agent B	2.5 ml
BSA Protein Standard (2mg/ml)	2.5 ml

### Storage Conditions:

Product is shipped at ambient temperature. Upon arrival, store Precipitation Reagent I and II at room temperature. Store Color Agent A at 2-8°C. Store Color Agent B at -20°C and protect from light. Store BSA Protein Standard at -20°C. When stored properly, the kit is stable for 1 year.

### Reagents that are Compatible with the Non-Interfering Protein Concentration Determination Kit:

P2-Mercaptoethanol, 15%	Ammonium Sulfate, 40%	Brij® 35, 1%	CHAPS, 4%	CHAPSO, 1%	Digitonin, 0.3%	DTT, 0.35M	EDTA, 0.1M
Glycerol, 30%	Guanidine Thiocyanate, 4M	Guanidine.HCl, 6M	HEPES, 0.1M	Hydrochloric acid, 0.1N	Imidazole, 0.5M	Iodoacetamide, 15mM	N-Octyl Glucosidase, 0.5%
Phosphate buffer, 0.2M	Sarcosyl, 1%	SDS, 2%	Sodium azide, 0.1M	Sodium Chloride, 0.5M	Sodium hydroxide, 2.5mM	Sucrose, 30%	TCEP, 15mM
Thesit, 2%	Thiourea, 2M	Tris.HCl, 0.5M	Triton® X-100, 3%	Triton® X-114, 3%	Tween® 20, 2%	Urea, 8M	Zwittergent® 3-12, 1.5%

### Prepare Agent-II:

Prior to use, prepare an appropriate volume of Agent II by mixing 100 parts of Color Agent A with 1 part of Color Agent B. E.g. For 10ml of Agent II, add 0.1ml Color Agent B to 10ml Color Agent A. Agent II can be stored refrigerated for one month or as long as the optical density of the solution at 475-490nm is less than 0.025 O.D.

## Protocols:

### A: Tube Test Assay:

1. Perform assay at room temperature and use 2ml tubes for assay.
2. Prepare a set of protein standards using the supplied BSA Protein Standard solution. For example, 0, 8, 16, 24, 40, 50µg.
3. Add 1-50µl of each protein sample to be assayed to separate 2ml tubes.
  - NOTE:** It is recommended that each sample be assayed in duplicate. The total amount of protein should not exceed 50µg and we recommend using various dilutions of each sample to ensure they are below 50µg.
  - NOTE:** For determination of protein concentration in buffers free of interfering agents, please skip steps 4-7.
4. Add 0.5ml Precipitation Reagent I to each tube and vortex. Incubate for 2-3 minutes at room temperature.
5. Add 0.5ml Precipitation Reagent II to the tubes and vortex.
6. Centrifuge the tubes at maximum speed (~10,000xg) for 5 minutes to pellet the precipitated protein. For easier identification of the pellet, ensure all the tubes are centrifuged with the cap hinge facing outwards.
7. Decant the supernatant and remove residual liquid with a pipette, then invert the tube on clean filter paper and allow the liquid to completely drain from the tube.
  - Optional:** To enhance washing for problematic samples, please see the Troubleshooting section.
8. Add 100µl Copper Solution (Agent I) and 400µl deionized water to each tube and vortex until the precipitated protein pellet dissolves.
9. Using a 1ml pipette, rapidly transfer 1ml Agent II directly into each tube and immediately mix by inverting the tubes.
10. Incubate at room temperature for 15-20 minutes and then immediately read absorbances at 480nm. The absorbance should be read within 10-20 minutes.
11. Plot absorbance against protein concentration to determine protein concentrations of each unknown.
  - NOTE:** Do not subtract blank reading from the sample reading as absorbance will decrease as protein concentration increases.

### B: Microplate Test Assay:

- NOTE:** For high throughput 96-well assays, we recommend using 2ml deep round or V- bottom well titer plates. Protocol requires centrifugation of the 96-well plate at 5,000xg and this may require a special centrifuge adaptor.
1. For high throughput 96-well assay, follow steps 1-5 of the above Tube Test Assay protocol.
  2. Centrifuge the 2ml deep round or V- bottom well titer plate at ~5,000xg for 7 minutes to pellet the precipitate. Discard the supernatant by inverting the titer plate. Allow the liquid to completely drain off the wells by placing the inverted titer plate on clean filter paper.
  3. Continue with the above Tube test assay protocol following steps 8-9.
  4. After incubation, transfer 200-250µl assay reaction product from each well into the corresponding well of a new flat bottom 96 well microplate and measure the absorbance at 480nm. The absorbance should be read within 10-20 minutes.
    - NOTE:** Do not subtract blank reading from the sample reading as absorbance will decrease as protein concentration increases.

## Troubleshooting:

Problems	Cause	Suggestions
Interference still occurring. (Readings very low and show limited change with increasing concentrations)	Interfering agents not fully removed due to carry over at step 7.	Ensure all supernatant is removed in step 7. There should be no residual liquid.
	Exceedingly high concentrations of interfering agents in starting samples.	If high concentrations of interfering agents are present, additional washing after step 7 may be required. Add 0.5ml Precipitation Reagent I and then 0.1ml Precipitation Reagent II to the pellet. Gently invert a few times without disturbing the precipitate and repeat steps 6-7.
A linear response between 0.5-50µg not visualized. The standard curve is not straight.	Poor centrifugation at Step 6. Failure to pellet all the protein.	Centrifuge for >5 minutes at >10,000xg.
	Insufficient mixing of Agent II	Use a 1ml pipette and rapidly shoot the Agent II directly into the tube. Immediately invert to mix.
Low protein concentrations.	Protein pellet not fully resuspended	Vortex pellet for 10-50 seconds at step 8.