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A Geno Technology, Inc. (USA) brand name

NI™ Protein Assay

A Non-Interfering™ Protein Assay

(Cat. # 786-005, 786-896)

INTRODUCTION

The Non-Interfering™ Protein Assay is a highly sensitive colorimetric assay that overcomes interference by common laboratory agents. The assay removes detergents (non-ionic, ionic and zwitterionic), reducing agents (β-mercaptoethanol, DTT), chelating agents (EDTA), amines (Tris), sugars, and is highly tolerant of strong chaotropic buffers.

The NI™ Protein Assay is suitable for determining protein concentrations in protein loading buffer (Laemmli buffer)[1-4], high β-mercaptoethanol concentrations (<15%)[5-7] and in lipid and vesicle preparations[8-9]. The NI™ Protein Assay has a linear response between 0.5-50µg and has a small sample requirement (1-50µl). The kit components are suitable for 500 assays.

ITEM(S) SUPPLIED

Description	Cat. # 786-005	Cat. # 786-896
UPPA™ I	250ml	250ml
UPPA™ II	250ml	250ml
Copper Solution (Reagent I)	50ml	50ml
Color Agent A	2 x 250ml	2 x 250ml
Color Agent B	5ml	5ml
BSA Protein Standard [2mg/ml]	5ml	-
Non-Animal Protein Standard [2mg/ml]	-	5ml

STORAGE CONDITIONS

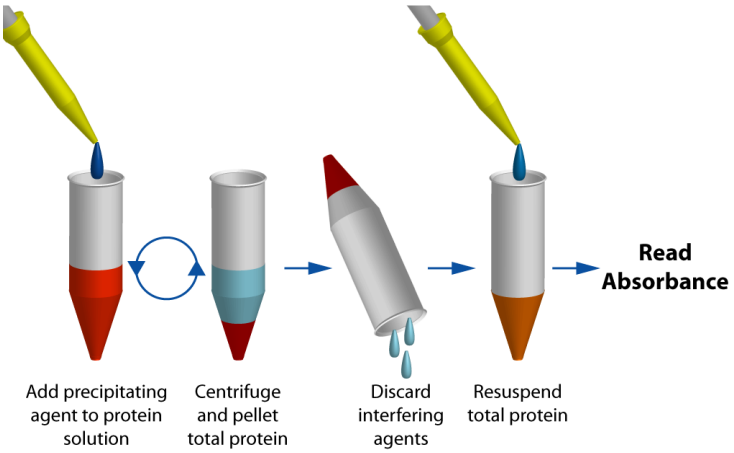
The kit is shipped at ambient temperature. Upon arrival, store UPPA-I and UPPA-II at room temperature. The remaining kit components should be stored in the dark and refrigerated in its original box. When stored properly, the kit is stable for 1 year.

OVERVIEW

The Non-Interfering™ Protein Assay is composed of two simple steps (see figure):

1. Universal Protein Precipitating Agent (UPPA™) is added to the protein solutions to rapidly precipitate total protein. Protein is immobilized by centrifugation and interfering agents in the supernatant are discarded.

2. Protein concentration is assayed by mixing with an alkaline solution containing a known concentration of copper salt; the copper ions bind to the peptide backbone and the assay measures the unbound copper ions. The assay is independent of protein side chains minimizing protein-to-protein variation. The color density is inversely proportional to the amount of protein.



TOLERANCE GUIDE

*Please note this assay is not compatible with solutions containing phenol red.

- 2-Mercaptoethanol, 15%
- Ammonium Sulfate, 40%
- Brij® 35, 1%
- CHAPS, 4%
- CHAPSO, 1%
- Digitonin, 0.3%
- DTT, 0.35M
- EDTA, 0.1M
- Glycerol, 30%
- Guanidine Thiocynate, 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

PREPARATION BEFORE USE

Prepare Reagent-II **-** Prior to use, prepare an appropriate volume of Reagent II by mixing 100 parts of Color Agent A with 1 part of Color Agent B. *(e.g. For 10ml of Reagent II, add 0.1ml Color Agent B to 10ml Color Agent A).*

Reagent 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.

PROTOCOL

1. Perform assays at room temperature. Use 2ml tubes for assay.
2. Prepare a set of protein standards using the supplied BSA or Non-Animal Protein Standard as indicated in the table below:

Tube #	**1**	**2**	**3**	**4**	**5**	**6**
Protein Standard [2mg/ml] (µl)	0	4	8	12	20	25
Protein (µg)	0	8	16	24	40	50

3. Add 1-50µl of the protein samples to be assayed to 2ml tubes.
   NOTE: It is recommended that duplicates are used. The total amount of protein should not exceed 50µg and we recommend various protein dilutions are used to ensure samples are below 50µg.
   NOTE: For determination of protein concentrations in buffers free of interfering agents skip steps 4-6.
4. Add 0.5ml UPPA™ I to each tube and vortex. Incubate for 2-3 minutes at room temperature.
5. Add 0.5ml UPPA™ 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. A small pellet should be visible.
7. Decant off the supernatant, return the tubes to the centrifuge as before, quickly pulse to spin down residual liquid and remove with a pipette.
   OPTIONAL: For enhanced washing for problematic samples see the Troubleshooting section.
8. Add 100µl Copper Solution (Reagent I) and 400µl deionized water to the tubes and vortex until the protein precipitate pellet dissolves.
9. Using 1ml pipette, rapidly shoot 1ml Reagent II directly into each tube containing Reagent I plus DI Water and immediately mix it by inverting the tubes.
10. Incubate at room temperature for 15-20 minutes and then immediately read absorbances at 480nm against DI water.
11. Plot absorbance against protein concentration and determine protein concentrations of unknowns.
    NOTE: *Do not subtract blank reading from the sample reading as absorbance will decrease as protein concentration increases.*

PROTOCOL FOR HIGH THROUGHPUT 96-WELL ASSAYS

NOTE: For high throughput 96-well assays, we recommend using 2ml deep round or V- bottom well titer plates. These are available from multiple sources, including VWR, Fisher and USA Scientific. The high throughput protocol requires centrifugation of the 96-well plate at 2-5,000xg and this may require a special centrifuge adaptor.

1. For high throughput 96-well assay, follow steps 1-5 of the above protocol.
2. Centrifuge the titer plate at ~5,000xg for 7 minutes to pellet the precipitate. Invert the titer plate to remove the supernatant and shake to remove all excess supernatant.
3. Continue with the above protocol following steps 8-10.
4. After incubation, transfer 200µl assay reaction to a flat bottom 96 well micro titer plate and measure the absorbances at 480nm against DI water.
5. Plot absorbance against protein concentration and determine protein concentrations of unknowns.

   NOTE: *Do not subtract blank reading from the sample reading as absorbance will decrease as protein concentration increases.*

TROUBLESHOOTING

Problem	**Reason**	**Solution**
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 UPPA I and then 0.1ml UPPA 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 Reagent II.	Use a 1ml pipette and rapidly shoot the Reagent II directly into the tube. Immediately invert to mix.
Low Protein Concentrations.	Protein pellet not fully resuspended	Vortex pellet for 10-30 seconds at step 8.

REFERENCES:

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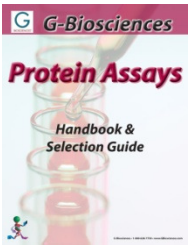

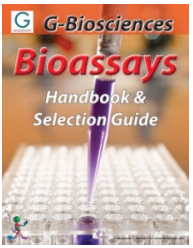

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