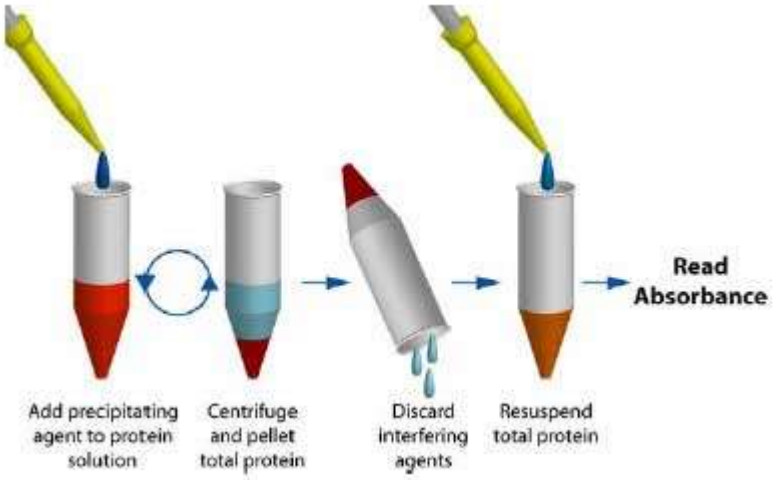
Revision	**01-November-2010 JSW**
Form	**500 Tests**
Format	**Cuvette or 2 ml, 96-deep-well plate**
Detection method	**Colorimetric**
Storage	**Upon arrival, store UPPA-I and UPPA-II at room temperature and the remaining components at 4°C, in the dark in the original box.**
Intended use	**The Non-Interfering 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. It is suitable for determining protein concentrations in protein loading buffer (Laemmeli buffer), high β-mercaptoethanol concentrations (<15%), and in lipid and vesicle preparations. The Non-Interfering Protein Assay™ is linear between 0.5-50 µg and requires a small sample (1-50 µl).**
Principles of the assay	**The Non-Interfering Protein Assay™ is composed of two simple steps. First, the Universal Protein Precipitating Agent (UPPA™) is added to the protein solutions to rapidly precipitate total protein. The protein is immobilized by centrifugation and interfering agents in the supernatant are discarded. Second, the protein concentration is assayed by mixing the sample 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 absorbance is inversely proportional to the amount of protein in the sample.**

	Figure 1: Non-Interfering™ Protein Assay Overview 
Materials provided	**The kit is supplied with enough reagents for 500 assays using Universal Protein Precipitating Agent (UPPA).** **• UPPA-I (Kit Component No. KP15001-250ML): 1 bottle, 250 ml** **• UPPA-II (Kit Component No. KP15002-250ML): 1 bottle, 250 ml** **• Copper Solution-Reagent-I (Kit Component No. KP15003-50ML): 1 bottle, 50 ml** **• Color Agent-A (Kit Component No. KP15004-250ML): 2 bottles, 250 ml each** **• Color Agent-B (Kit Component No. KP15005-5ML): 1 vial, 5 ml** **• Protein Standard BSA (2 mg/ml) (Kit Component No. KP15006-5ML): 1 vial, 5 ml**
Materials Required but not provided	**• 2 ml tubes**
Precautions and recommendations	**• Always use clean, sterile pipets and aseptic techniques for removing reagents from the reagent bottles.**

<table>
<tr><td>Reagent preparation</td><td>• Reagent II: Prior to use, prepare an appropriate volume of Reagent II by mixing 100 parts Color Agent-A with 1 part Color Agent-B. Mark this solution as Reagent II. Fresh or unused Reagent II can be stored at 4°C and is stable for up to 1 month or as long as the absorbance of the solution remains below 0.025 at 480 nm.</td></tr>
<tr><td>Detailed protocol</td><td>Perform the assay at room temperature.
1. Prepare a set of protein standards using the supplied BSA Protein Standard as indicated in the table below:

Table 1: Set of Protein Standards

<table>
<tr><td>Tube #</td><td>1</td><td>2</td><td>3</td><td>4</td><td>5</td><td>6</td></tr>
<tr><td>BSA Protein Std [2mg/ml] (µl)</td><td>0</td><td>4</td><td>8</td><td>12</td><td>20</td><td>25</td></tr>
<tr><td>Protein (µg)</td><td>0</td><td>8</td><td>16</td><td>24</td><td>40</td><td>50</td></tr>
</table>

2. Add 1-50 µl of unknown protein samples to 2 ml tubes.

NOTE: It is recommended that samples be assayed in duplicate. The total amount of protein should not exceed 50 µg, so it is recommended that several dilutions of samples be assayed to ensure that samples are below 50 µg.
NOTE: For determination of protein concentrations in buffers free of interfering agents skip steps 3-6 (i.e., do not add UPPA-I or UPPA-II if your buffer does not contain any interfering agents).

3. Add 500 µl UPPA™ I to each tube and vortex. Incubate for 2-3 min at room temperature.
4. Add 500 µl UPPA™ II to the tubes and vortex.
5. Centrifuge the tubes at maximum speed (~10,000 x g) for 5 min 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.
6. Decant the supernatant, return the tubes to the centrifuge, pulse to centrifuge the residual liquid, and remove with a pipette.
OPTIONAL: For enhanced washing for problematic samples see</td></tr>
</table>

	the Troubleshooting section. **7. Add 100 µl Copper Solution (Reagent I) and 400 µl deionized water to the tubes and vortex until the protein precipitate pellet dissolves.** **8. Using a 1-ml pipette, rapidly shoot 1 ml Reagent II directly into each tube containing Copper Solution (Reagent I) plus diH_2O and immediately mix by inverting the tubes.** **11. Incubate at room temperature for 15-20 min and then immediately read absorbances at 480 nm against diH_2O.** **10. Plot the absorbance against the protein concentration and determine protein concentrations of unknowns by comparing to the standard curve. 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 2 ml deep round or V- bottom well titer plates. The high throughput protocol requires centrifugation of the 96-well plate at 2-5000 x *g* and this may require a special centrifuge adaptor.** **1. For performing the high throughput, in a 96-well format, follow steps 1-5 of the above protocol.** **2. Centrifuge the plate at ~5000 x *g* for 7 min to pellet the precipitate. Invert the 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 200 µl assay reaction to a flat bottom 96-well plate and measure the absorbance at 480 nm against DI water.** **5. Plot the 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.**

Limitations of the assay	**Table 2: Tolerance Guide** Tolerance Guide: The Non-Interfering Protein Assay™ has been successfully used with the following reagents at the indicated concentrations: ▪ 2-Mercaptoethanol, 15% ▪ Ammonium Sulfate, 40% ▪ Brij® detergent, 1% ▪ CHAPS, 4% ▪ CHAPSO, 1% ▪ Digitonin, 0.3% ▪ DTT, 0.35 M ▪ EDTA, 0.1 M ▪ Glycerol, 30% ▪ Guanidine thiocyanate, 4 M ▪ Guanidine HCl, 6 M ▪ HEPES, 0.1 M ▪ Hydrochloric acid, 0.1 N ▪ Imidazole, 0.5 M ▪ Iodoacetamide, 15 mM ▪ N-octyl Glucosidase, 0.5% ▪ Phosphate buffer, 0.2 M ▪ Sarcosyl, 1% ▪ SDS, 2% ▪ Sodium azide, 0.1 M ▪ Sodium Chloride, 0.5 M ▪ Sodium hydroxide, 2.5 mM ▪ Sucrose, 30% ▪ TCEP, 15 mM ▪ Thesit, 2% ▪ Thiourea, 2 M ▪ Tris-HCl, 0.5 M ▪ Triton® X-100 detergent, 3% ▪ Triton® X-114 detergent, 3% ▪ Tween® 20 detergent, 2% ▪ Urea, 8 M ▪ Zwittergent® 3-12 detergent, 1.5%
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