Introduction

The Thermo Scientific™ Micro BCA™ Protein Assay Kit is a detergent-compatible bicinchoninic acid formulation for the colorimetric detection and quantitation of total protein. An adaptation of the Thermo Scientific™ BCA Protein Assay Kit (Product No. 23225), the Micro BCA Kit has been optimized for use with dilute protein samples (0.5-20µg/mL). The unique, patented method uses bicinchoninic acid (BCA) as the detection reagent for Cu⁺¹, which is formed when Cu⁺² is reduced by protein in an alkaline environment.¹ A purple-colored reaction product is formed by the chelation of two molecules of BCA with one cuprous ion (Cu⁺¹). This water-soluble complex exhibits a strong absorbance at 562nm that is linear with increasing protein concentrations.

The macromolecular structure of protein, the number of peptide bonds and the presence of four amino acids (cysteine, cystine, tryptophan and tyrosine) are reported to be responsible for color formation with BCA.² Studies with di-, tri- and tetrapeptides suggest that the extent of color formation is caused by more than the mere sum of individual color-producing functional groups.²

The Micro BCA Protein Assay Kit uses concentrated reagents and a protocol that utilizes an extended incubation time at an elevated temperature (60°C, Test Tube Procedure only). The result is an extremely sensitive colorimetric protein assay in a test tube or microplate assay format.

Note: For peptide sample concentration measurements, use the Thermo Scientific™ Pierce™ Quantitative Fluorometric Peptide Assay or the Pierce™ Quantitative Colorimetric Peptide Assay (see Related Thermo Scientific Products).
Preparation of Standards and Working Reagent

A. Preparation of Diluted Albumin (BSA) Standards

Use Table 1 as a guide to prepare a set of protein standards. Dilute the contents of one Albumin (BSA) Standard ampule into several clean vials, preferably using a diluent that is similar to the sample buffer. Each 1mL ampule of 2.0mg/mL Albumin Standard is sufficient to prepare a set of diluted standards such that three replicates of each dilution may be included in the Test Tube Procedure.

Table 1. Preparation of Diluted Albumin (BSA) Standards

<table>
<thead>
<tr>
<th>Vial</th>
<th>Volume of Diluent</th>
<th>Volume and Source of BSA</th>
<th>Final BSA Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>4.5mL</td>
<td>0.5mL of Stock</td>
<td>200µg/mL</td>
</tr>
<tr>
<td>B</td>
<td>8.0mL</td>
<td>2.0mL of vial A dilution</td>
<td>40µg/mL</td>
</tr>
<tr>
<td>C</td>
<td>4.0mL</td>
<td>4.0mL of vial B dilution</td>
<td>20µg/mL</td>
</tr>
<tr>
<td>D</td>
<td>4.0mL</td>
<td>4.0mL of vial C dilution</td>
<td>10µg/mL</td>
</tr>
<tr>
<td>E</td>
<td>4.0mL</td>
<td>4.0mL of vial D dilution</td>
<td>5µg/mL</td>
</tr>
<tr>
<td>F</td>
<td>4.0mL</td>
<td>4.0mL of vial E dilution</td>
<td>2.5µg/mL</td>
</tr>
<tr>
<td>G</td>
<td>4.8mL</td>
<td>3.2mL of vial F dilution</td>
<td>1µg/mL</td>
</tr>
<tr>
<td>H</td>
<td>4.0mL</td>
<td>4.0mL of vial G dilution</td>
<td>0.5µg/mL</td>
</tr>
<tr>
<td>I</td>
<td>8.0mL</td>
<td>0</td>
<td>0µg/mL = Blank</td>
</tr>
</tbody>
</table>

B. Preparation of the Micro BCA Working Reagent (WR)

1. Use the following formula to determine the total volume of WR required:

   \[
   (\# \text{ standards} + \# \text{ unknowns}) \times (\# \text{ replicates}) \times (\text{volume of WR per sample}) = \text{total volume WR required}
   \]

   Example: for the standard Test Tube Procedure with 3 unknowns and 2 replicates of each sample:

   \[
   (9 \text{ standards} + 3 \text{ unknowns}) \times (2 \text{ replicates}) \times (1\text{mL}) = 24\text{mL WR required (round up to 25mL)}
   \]

   Note: 1mL of the WR is required for each sample in the Test Tube Procedure, while only 150µL of WR is required for each sample in the Microplate Procedure.

2. Prepare WR by mixing 25 parts of Micro BCA Reagent MA and 24 parts Reagent MB with 1 part of Reagent MC (25:24:1, Reagent MA:MB:MC). For the above example, combine 12.5mL of Reagent MA and 12.0mL Reagent MB with 0.5mL of Reagent MC.

   Note: When Reagent MC is initially added to Reagents MA and MB, turbidity occurs that quickly disappears upon mixing to yield a clear-green solution. Prepare sufficient volume of WR based on the number of samples to be assayed. The WR is stable for one day when stored in a closed container at room temperature (RT). It is not necessary to protect the solution from light.

Procedure Summary (Test Tube Procedure)

50 parts “MA”
48 parts “MB”
2 parts “MC”

1.0 ml Sample + 1.0 ml Working Reagent
Incubate: 60 min. at 60°C
Spectrophotometer

Mixed working reagent → Mix well → Then cool → Read at 562 nm
**Test Tube Procedure (linear working range of 0.5-20µg/mL)**

1. Pipette 1.0mL of each standard and unknown sample replicate into appropriately labeled test tubes.
2. Add 1.0mL of the WR to each tube and mix well.
3. Cover tubes and incubate at 60°C in a water bath for 1 hour.
4. Cool all tubes to room temperature (RT).
5. With the spectrophotometer set to 562nm, zero the instrument on a cuvette filled only with water. Subsequently, measure the absorbance of all the samples within 10 minutes.
   
   **Note:** Color development continues even after cooling to RT. However, the rate of development at RT is sufficient low that no significant error is introduced if all absorbance measurements are made within a 10-minute period.
6. Subtract the average 562nm absorbance reading of the Blank standard replicates from the 562nm reading of all other individual standard and unknown sample replicates.
7. Prepare a standard curve by plotting the average Blank-corrected 562nm reading for each BSA standard vs. its concentration in µg/mL. Use the standard curve to determine the protein concentration of each unknown sample.

**Microplate Procedure (linear working range of 2-40µg/mL)**

1. Pipette 150µL of each standard or unknown sample replicate into a microplate well (Product No. 15041).
2. Add 150µL of the WR to each well and mix plate thoroughly on a plate shaker for 30 seconds.
3. Cover plate using Sealing Tape for 96-Well Plates (Product No. 15036) and incubate at 37°C for 2 hours.
   
   **Note:** Limit incubations of microplate to less than or equal to 37°C, otherwise high background and aberrant color development may result. Most polystyrene assay plates deform, leach, and become cloudy at 60°C.
4. Cool plate to room temperature (RT).
5. Measure the absorbance at or near 562nm on a plate reader.
6. Subtract the average 562nm absorbance reading of the Blank standard replicates from the 562nm reading of all other individual standard and unknown sample replicates.
7. Prepare a standard curve by plotting the average Blank-corrected 562nm reading for each BSA standard vs. its concentration in µg/mL. Use the standard curve to determine the protein concentration of each unknown sample.

**Troubleshooting**

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>No color in any tubes</td>
<td>Sample contains a copper chelating agent</td>
<td>Dialyze, desalt, or dilute sample Increase copper concentration in working reagent (e.g., use more Reagent MC)</td>
</tr>
<tr>
<td>Blank absorbance is OK, but standards and samples show less color than expected</td>
<td>Strong acid or alkaline buffer, alters working reagent pH</td>
<td>Dialyze, desalt, or dilute sample</td>
</tr>
<tr>
<td>Color of samples appears darker than expected</td>
<td>Protein concentration is too high</td>
<td>Dilute sample</td>
</tr>
<tr>
<td>All tubes (including blank) are dark purple</td>
<td>Buffer contains a reducing agent Buffer contains a thiol Buffer contains biogenic amines (catecholamines)</td>
<td>Dialyze or dilute sample</td>
</tr>
<tr>
<td>Need to measure color at a different wavelength</td>
<td>Spectrophotometer or plate reader does not have 562nm filter</td>
<td>Wavelengths between 540nm and 590nm can be used, but standard curve slope and overall assay sensitivity will be decreased. See Tech Tip on website</td>
</tr>
</tbody>
</table>
Additional Information

A. Interfering Substances

Certain substances are known to interfere with the Micro BCA Assay including those with reducing potential, chelating agents, and strong acids or bases. Avoid the following substances as components of the sample buffer:

<table>
<thead>
<tr>
<th>Substance</th>
<th>Substance</th>
<th>Substance</th>
<th>Substance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic Acid</td>
<td>Hydrogen Peroxide</td>
<td>Iron</td>
<td>Reducing Sugars</td>
</tr>
<tr>
<td>Catecholamines</td>
<td>Hydrazides</td>
<td>Lipids</td>
<td>Tryptophan</td>
</tr>
<tr>
<td>Cysteine</td>
<td>Impure Glycerol</td>
<td>Phenol Red</td>
<td>Tyrosine</td>
</tr>
<tr>
<td>EGTA</td>
<td>Impure Sucrose</td>
<td>Reducing Agents</td>
<td>Uric Acid</td>
</tr>
</tbody>
</table>

Maximum compatible concentrations for many substances in the Test Tube Procedure are listed in Table 2 (see last page). Substances were considered compatible at the indicated concentration if the error in protein concentration estimation caused by the presence of the substance in the sample was less than or equal to 10%. The substances were tested using WR prepared immediately before each experiment. The Blank-corrected 562nm absorbance measurements (for the 10µg/mL BSA standard + substance) were compared to the net 562nm readings of the same standard prepared in 0.9% saline.

B. Strategies for Eliminating or Minimizing the Effects of Interfering Substances

The effects of interfering substances in the Micro BCA Protein Assay may be eliminated or overcome by several methods.

- Remove the interfering substance by dialysis or gel filtration (see Related Thermo Scientific Products).
- Dilute the sample until the substance no longer interferes. (This is only effective for relatively concentrated samples.)
- Precipitate proteins with acetone or trichloroacetic acid (TCA).4
- Increase the amount of copper in the WR (prepare WR using a greater proportion of Reagent MC; e.g., MA:MB:MC equal to 25:24:2 or 25:24:3), which may eliminate interference by copper chelating agents.

Note: For greatest accuracy, the protein standards must be treated identically to the sample(s).

C. Response Characteristics for Different Proteins

Each of the commonly used total protein assay methods exhibits some degree of varying response toward different proteins (Table 3). These differences relate to amino acid sequence, pI, structure and the presence of certain side chains or prosthetic groups that can dramatically alter the protein’s color response. Most protein assay methods use BSA or immunoglobulin (IgG) as the standard against which the concentration of protein in the sample is determined. Pierce Albumin Standard (BSA) (Product No. 23209) provides a consistent standard for protein estimations. Nevertheless, individual proteins, including BSA and IgG, differ slightly in their color responses in the Micro BCA Assay (Figure 1). Therefore, for maximum accuracy use a purified (known concentration) sample of the target protein as the assay standard.

![Figure 1. Typical color response curves for BSA and BGG using the Test Tube Procedure.](image)

Table 3. Protein-to-Protein Variation. Absorbance ratios (562nm) for proteins relative to BSA using the Test Tube Procedure.

<table>
<thead>
<tr>
<th>Protein Tested</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin, bovine serum</td>
<td>1.00</td>
</tr>
<tr>
<td>Aldolase, rabbit muscle</td>
<td>0.80</td>
</tr>
<tr>
<td>α-Chymotrypsinogen, bovine</td>
<td>0.99</td>
</tr>
<tr>
<td>Cytochrome C, horse heart</td>
<td>1.11</td>
</tr>
<tr>
<td>Gamma globulin, bovine</td>
<td>0.95</td>
</tr>
<tr>
<td>IgG, bovine</td>
<td>1.12</td>
</tr>
<tr>
<td>IgG, human</td>
<td>1.03</td>
</tr>
<tr>
<td>IgG, mouse</td>
<td>1.23</td>
</tr>
<tr>
<td>IgG, rabbit</td>
<td>1.12</td>
</tr>
<tr>
<td>IgG, sheep</td>
<td>1.14</td>
</tr>
<tr>
<td>Insulin, bovine pancreas</td>
<td>1.22</td>
</tr>
<tr>
<td>Myoglobin, horse heart</td>
<td>0.92</td>
</tr>
<tr>
<td>Ovalbumin</td>
<td>1.08</td>
</tr>
<tr>
<td>Transferrin, human</td>
<td>0.98</td>
</tr>
</tbody>
</table>

| Average Ratio | 1.05 |
| Standard Deviation | 0.12 |
| Coefficient of Variation | 11.4% |
D. Alternative Total Protein Assay Reagents

If interference by a reducing substance or metal-chelating substance contained in the sample cannot be overcome, try the Thermo Scientific™ Coomassie Plus™ (Bradford) Protein Assay Kit (Product No. 23236), which is less sensitive to such substances.

E. Cleaning and Re-using Glassware

Care must be exercised when re-using glassware. The Micro BCA WR is sensitive to metal ions, especially copper ions. All glassware must be cleaned and then given a thorough final rinse with ultrapure water.

Related Thermo Scientific Products

<table>
<thead>
<tr>
<th>Code</th>
<th>Product Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>15041</td>
<td>Pierce™ 96-Well Plates, 100/pkg</td>
</tr>
<tr>
<td>15075</td>
<td>Reagent Reservoirs, 50mL, 200/pkg</td>
</tr>
<tr>
<td>15036</td>
<td>Sealing Tape for 96-Well Plates, 100/pkg</td>
</tr>
<tr>
<td>23209</td>
<td>Bovine Serum Albumin (BSA) Standard Ampules, 2mg/mL, 10 × 1mL</td>
</tr>
<tr>
<td>23212</td>
<td>Bovine Gamma Globulin (BGG) Standard Ampules, 2mg/mL, 10 × 1mL</td>
</tr>
<tr>
<td>23290</td>
<td>Pierce Quantitative Fluorometric Peptide Assay</td>
</tr>
<tr>
<td>23275</td>
<td>Pierce Quantitative Colorimetric Peptide Assay</td>
</tr>
<tr>
<td>23236</td>
<td>Coomassie Plus™ (Bradford) Protein Assay Kit</td>
</tr>
<tr>
<td>89882</td>
<td>Zeba™ Spin Desalting Columns, 0.5mL</td>
</tr>
<tr>
<td>89889</td>
<td>Zeba Spin Desalting Columns, 2mL</td>
</tr>
<tr>
<td>69576</td>
<td>Slide-A-Lyzer™ MINI Dialysis Units</td>
</tr>
<tr>
<td>69576</td>
<td>Slide-A-Lyzer Dialysis Cassettes</td>
</tr>
</tbody>
</table>

Cited References


General References


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Table 2. Compatible Substance Concentrations in the Micro BCA Protein Assay (see text for details).

<table>
<thead>
<tr>
<th>Substance</th>
<th>Salts/Buffers</th>
<th>Compatible Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACES, pH 7.8</td>
<td>10mM</td>
<td></td>
</tr>
<tr>
<td>Ammonium sulfate</td>
<td>2mM</td>
<td></td>
</tr>
<tr>
<td>Bicine, pH 8.4</td>
<td>0.2mM</td>
<td></td>
</tr>
<tr>
<td>Bis-Tris, pH 6.5</td>
<td>1:4 dilution*</td>
<td></td>
</tr>
<tr>
<td>Borate (50mM), pH 8.5 (#28384)</td>
<td>1:10 dilution*</td>
<td></td>
</tr>
<tr>
<td>B-Per™ Reagent (#78248)</td>
<td>Calcium chloride in TBS, pH 7.2</td>
<td>10mM</td>
</tr>
<tr>
<td>Na-Carbonate/Na-Bicarbonate (0.2 M), pH 9.4 (#28382)</td>
<td>undiluted</td>
<td></td>
</tr>
<tr>
<td>CES, pH 9.0</td>
<td>100mM</td>
<td></td>
</tr>
<tr>
<td>Na-Citrate (0.6 M), Na-Carbonate (0.1 M), pH 9.0 (#28388)</td>
<td>1:600 dilution*</td>
<td></td>
</tr>
<tr>
<td>Na-Citrate (0.6 M), MOPS (0.1 M), pH 7.5 (#28386)</td>
<td>1:600 dilution*</td>
<td></td>
</tr>
<tr>
<td>Cobalt chloride in TBS, pH 7.2</td>
<td>undiluted</td>
<td></td>
</tr>
<tr>
<td>EPIS, pH 8.0</td>
<td>100mM</td>
<td></td>
</tr>
<tr>
<td>Ferric chloride in TBS, pH 7.2</td>
<td>0.5mM</td>
<td></td>
</tr>
<tr>
<td>Glycine</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>Guanidine-HCl</td>
<td>4M</td>
<td></td>
</tr>
<tr>
<td>HEPES, pH 7.5</td>
<td>100mM</td>
<td></td>
</tr>
<tr>
<td>Imidazole, pH 7.0</td>
<td>12.5mM</td>
<td></td>
</tr>
<tr>
<td>MES, pH 6.1</td>
<td>100mM</td>
<td></td>
</tr>
<tr>
<td>MES (0.1M), NaCl (0.9%), pH 4.7 (#28390)</td>
<td>1:4 dilution*</td>
<td></td>
</tr>
<tr>
<td>MOPS, pH 7.2</td>
<td>100mM</td>
<td></td>
</tr>
<tr>
<td>Modified Dulbecco’s PBS, pH 7.4 (#28374)</td>
<td>0.2mM</td>
<td></td>
</tr>
<tr>
<td>Nickel chloride in TBS, pH 7.2</td>
<td>undiluted</td>
<td></td>
</tr>
<tr>
<td>PBS, Phosphate (0.1M), NaCl (0.15M), pH 7.2 (#28372)</td>
<td>100mM</td>
<td></td>
</tr>
<tr>
<td>PIPES, pH 6.8</td>
<td>100mM</td>
<td></td>
</tr>
<tr>
<td>RIPA lysis buffer, 50mM Tris, 150mM NaCl, 0.5% DOC, 1% NP-40, 0.1% SDS, pH 8.0</td>
<td>1:10 dilution*</td>
<td></td>
</tr>
<tr>
<td>Sodium acetate, pH 4.8</td>
<td>200mM</td>
<td></td>
</tr>
<tr>
<td>Sodium azide</td>
<td>0.2%</td>
<td></td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>100mM</td>
<td></td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>1M</td>
<td></td>
</tr>
<tr>
<td>Sodium citrate, pH 4.8 (or pH 6.4)</td>
<td>5M (16.7mM)</td>
<td></td>
</tr>
<tr>
<td>Sodium phosphate</td>
<td>100mM</td>
<td></td>
</tr>
<tr>
<td>Tricine, pH 8.0</td>
<td>2.5mM</td>
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<tr>
<td>Trisethanolamine, pH 7.8</td>
<td>0.5mM</td>
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</tr>
<tr>
<td>Tris</td>
<td>50mM</td>
<td></td>
</tr>
<tr>
<td>TBS; Tris (25mM), NaCl (0.15M), pH 7.6 (#28376)</td>
<td>1:10 dilution*</td>
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<tr>
<td>Tris (25mM), Glycine (192mM), pH 8.0 (#28380)</td>
<td>1:10 dilution*</td>
<td></td>
</tr>
<tr>
<td>Tris (25mM), Glycine (192mM), SDS (0.1%), pH 8.3 (#28378)</td>
<td>undiluted</td>
<td></td>
</tr>
<tr>
<td>Zinc chloride in TBS, pH 7.2</td>
<td>0.5mM</td>
<td></td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Substance</th>
<th>Detergents**</th>
<th>Compatible Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brij™-35</td>
<td>5.0%</td>
<td></td>
</tr>
<tr>
<td>Brij-56, Brij-58</td>
<td>1.0%</td>
<td></td>
</tr>
<tr>
<td>CHAPS (CHAPSO)</td>
<td>1.0% (5.0%)</td>
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</tr>
<tr>
<td>Deoxycholic acid</td>
<td>5.0%</td>
<td></td>
</tr>
<tr>
<td>Nonidet P-40 (NP-40)</td>
<td>5.0%</td>
<td></td>
</tr>
<tr>
<td>Octyl β-glucoside</td>
<td>0.1%</td>
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</tr>
<tr>
<td>Octyl β-thioglucopyranoside</td>
<td>5.0%</td>
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</tr>
<tr>
<td>SDS</td>
<td>5.0%</td>
<td></td>
</tr>
<tr>
<td>Span™ 20</td>
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</tr>
<tr>
<td>Triton™ X-100</td>
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<tr>
<td>Triton X-114</td>
<td>0.05%</td>
<td></td>
</tr>
<tr>
<td>Triton X-305, X-405</td>
<td>1.0%</td>
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</tr>
<tr>
<td>Tween™-20, Tween-80</td>
<td>5.0%</td>
<td></td>
</tr>
<tr>
<td>Tween-60</td>
<td>0.5%</td>
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</tr>
<tr>
<td>Zwittergent™ 3-14</td>
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<table>
<thead>
<tr>
<th>Substance</th>
<th>Chelating agents</th>
<th>Compatible Concentration</th>
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</thead>
<tbody>
<tr>
<td>EDTA</td>
<td>0.5mM</td>
<td></td>
</tr>
<tr>
<td>EGTA</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>Sodium citrate, pH 4.8 (or pH 6.4)</td>
<td>5mM (16.7mM)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Substance</th>
<th>Reducing &amp; Thiol-Containing Agents</th>
<th>Compatible Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-acetylgalactosamine in PBS, pH 7.2</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>Cysteine</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>Dithioerythritol (DTE)</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>Dithiothreitol (DTT)</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>1mM</td>
<td></td>
</tr>
<tr>
<td>2-Mercaptoethanol</td>
<td>1mM</td>
<td></td>
</tr>
<tr>
<td>Thimerosal</td>
<td>---</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Substance</th>
<th>Misc. Reagents &amp; Solvents</th>
<th>Compatible Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone</td>
<td>1.0%</td>
<td></td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>1.0%</td>
<td></td>
</tr>
<tr>
<td>Aprotinin</td>
<td>1mg/L</td>
<td></td>
</tr>
<tr>
<td>DMF, DMSO</td>
<td>1.0%</td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td>1.0%</td>
<td></td>
</tr>
<tr>
<td>Glycerol (Fresh)</td>
<td>1.0%</td>
<td></td>
</tr>
<tr>
<td>Hydrazide</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>Hydrides (Na2BH4 or NaCNBH3)</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>Hydrochloric Acid</td>
<td>10mM</td>
<td></td>
</tr>
<tr>
<td>Leupeptin</td>
<td>10mg/L</td>
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</tr>
<tr>
<td>Methanol</td>
<td>1.0%</td>
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</tr>
<tr>
<td>Phenol Red</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>PMSF</td>
<td>1mM</td>
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</tr>
<tr>
<td>Sodium Hydroxide</td>
<td>50mM</td>
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</tr>
<tr>
<td>Sucrose</td>
<td>4%</td>
<td></td>
</tr>
<tr>
<td>TLCK</td>
<td>0.1mg/L</td>
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</tr>
<tr>
<td>TPCK</td>
<td>0.1mg/L</td>
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</tr>
<tr>
<td>Urea</td>
<td>3M</td>
<td></td>
</tr>
<tr>
<td>o-Vanadate (sodium salt), in PBS, pH 7.2</td>
<td>1mM</td>
<td></td>
</tr>
</tbody>
</table>

* Diluted with ultrapure water
** Detergents were tested using high-purity Thermo Scientific™ Surfact-Amps™ Products, which have low peroxide content
--- Dashed-line entry indicates that the material is incompatible with the assay