**Setting Up a Standard Curve**

Determine protein concentration by plotting the absorbance vs. concentration of known standards. Use the resulting curve to determine the concentration of unknown proteins based on their absorbance.

**Note:** The best standard to use is a purified sample of your target protein. If this is not available, use Bio-Rad's bovine serum albumin or bovine γ-globulin to make your standard curve.

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**For Best Results**

- Run a standard curve with each protein assay
- Run at least 3 replicates of all standards and samples
- Process the sample and standards the same way to ensure that differences in color intensity are due only to differences in protein concentration
- Make sure the sample and standard fall within the same concentration range

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**Standard curve generation using known standards.** A typical standard curve for Lowry-based assays, including RC DC Protein Assays. Bovine serum albumin (a); bovine γ-globulin (A).

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**Lowry-based**: reductant and detergent compatible

Reagent Compatibility
The following reagents were tested for their compatibility with the protein assay. Concentrations represent maximum concentrations for standard assay. ✔ = compatible.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHAPS</td>
<td>2%</td>
</tr>
<tr>
<td>Dithiothreitol (DTT)</td>
<td>0.1 M</td>
</tr>
<tr>
<td>EDTA</td>
<td>0.1 M</td>
</tr>
<tr>
<td>Imidazole</td>
<td>0.5 M</td>
</tr>
<tr>
<td>Laemmli buffer</td>
<td>✔</td>
</tr>
<tr>
<td>2-Mercaptoethanol</td>
<td>5%</td>
</tr>
<tr>
<td>Sodium hydroxide</td>
<td>2.5 M</td>
</tr>
<tr>
<td>TBP</td>
<td>0.002 M</td>
</tr>
<tr>
<td>Tris, pH 8</td>
<td>0.5 M</td>
</tr>
<tr>
<td>Triton X-100</td>
<td>2%</td>
</tr>
<tr>
<td>Tween 20</td>
<td>2%</td>
</tr>
</tbody>
</table>

Ordering Information

Catalog #          Description
5000121            RC DC Protein Assay Kit I, includes RC reagents package, DC protein assay reagents package, bovine γ-globulin standard
5000122            RC DC Protein Assay Kit II, includes RC reagents package, DC protein assay reagents package, bovine serum albumin standard

Microplate Reader
1681130            iMark™ Microplate Absorbance Reader
2240096            Costar 96-Well Flat-Bottom EIA Plate, 100 plates

Spectrophotometer
1702505            SmartSpec™ Plus Spectrophotometer
1702510            iTruView™ Cuvettes, pkg of 50
1702511            iTruView Cuvettes, pkg of 100
2239950            Standard Disposable Polystyrene Cuvettes, 3.5 ml, pkg of 100
2239955            Semimicrovolume Disposable Polystyrene Cuvettes, 1.5 ml, pkg of 100
1702502            Standard Cuvette, 1–3.5 ml, quartz
1702503            Semimicrovolume Cuvette, 0.6–1.4 ml, quartz
1702504            Microvolume Cuvette, 200–700 μl, quartz
1702505            Submicrovolume Cuvette, 80–150 μl, quartz

Visit bio-rad.com/proteinassaykits for more information.

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