

## Compatibility of Promega Lysis Buffer with Reporter and Protein Assays

Lysis Buffer	Compatible Reporter Assay	Protein Assay (4)	Western Blot (5)
<b>Cell Culture Lysis Reagent (CCLR)</b>	<ol style="list-style-type: none"> <li>1. Luciferase Assay System (1)</li> <li>2. <math>\beta</math>-galactosidase assay (<math>\beta</math>-Galactosidase Enzyme Assay System with Reporter Lysis Buffer) (2)</li> </ol>	<ul style="list-style-type: none"> <li>• GenoTechnology NI™ Protein Assay</li> <li>• Pierce BCA Protein Assay with Compat-Able™ Protein Assay Preparation Reagent Set</li> <li>• Bio-Rad RC DC™ Protein Assay</li> <li>• Molecular Probes NanoOrange® Protein Quantitation Kit (dilute lysate 1:10)</li> <li>• BioRad DC Protein Assay or Pierce BCA Protein Assay (dilute lysate at least 1:2)</li> </ul>	<ul style="list-style-type: none"> <li>• Compatible</li> <li>• Can release cytoplasmic, mitochondrial and nuclear contents (6)</li> </ul>
<b>Reporter Lysis Buffer (RLB)</b>	<ol style="list-style-type: none"> <li>1. Luciferase Assay System (1)</li> <li>2. <math>\beta</math>-galactosidase assay (including both <math>\beta</math>-Galactosidase Enzyme Assay System with Reporter Lysis Buffer and Beta-Glo® Assay System)</li> <li>3. CAT Enzyme Assay System with Reporter Lysis Buffer</li> </ol>	<ul style="list-style-type: none"> <li>• GenoTechnology NI™ Protein Assay</li> <li>• Pierce BCA Protein Assay with Compat-Able™ Protein Assay Preparation Reagent Set</li> <li>• Pierce Coomassie® Plus protein assay</li> <li>• Bio-Rad RC DC™ Protein Assay</li> <li>• Normal Bradford assay with 2–8<math>\mu</math>l of undiluted lysate</li> <li>• Molecular Probes NanoOrange® Protein Quantitation Kit (dilute lysate 1:10)</li> <li>• BioRad DC Protein Assay (large scale only) or Pierce BCA Protein Assay (dilute lysate at least 1:2)</li> </ul>	<ul style="list-style-type: none"> <li>• Compatible</li> <li>• Can release cytoplasmic and nuclear contents. RLB releases nuclear contents more efficiently with a freeze-thaw cycle (6)</li> </ul>
<b>Passive Lysis Buffer (PLB)</b>	<ol style="list-style-type: none"> <li>1. Luciferase Assay System (1)</li> <li>2. <math>\beta</math>-galactosidase assay (including both <math>\beta</math>-Galactosidase Enzyme Assay System with Reporter Lysis Buffer and Beta-Glo® Assay System)</li> <li>3. CAT Enzyme Assay System with Reporter Lysis Buffer</li> <li>4. Dual-Luciferase® Reporter Assay</li> </ol>	<ul style="list-style-type: none"> <li>• GenoTechnology NI™ Protein Assay</li> <li>• Pierce BCA Protein Assay with Compat-Able™ Protein Assay Preparation Reagent Set</li> <li>• Pierce Coomassie® Plus protein assay</li> <li>• Bio-Rad RC DC™ Protein Assay</li> <li>• Pierce BCA Protein Assay (dilute lysate 1:10)</li> <li>• Molecular Probes NanoOrange® Protein Quantitation Kit (dilute lysate 1:10)</li> </ul>	<ul style="list-style-type: none"> <li>• Compatible</li> <li>• Can release cytoplasmic and nuclear contents (6)</li> </ul>
<b>Glo Lysis Buffer (GLB)</b>	<ol style="list-style-type: none"> <li>1. Luciferase Assay System (1)</li> <li>2. Bright-Glo™ Luciferase Assay and Steady-Glo® Luciferase Assay</li> <li>3. ONE-Glo™ Luciferase Assay System</li> <li>4. <math>\beta</math>-galactosidase assay (including both <math>\beta</math>-Galactosidase Enzyme Assay System with Reporter Lysis Buffer and Beta-Glo® Assay System) (up to 45<math>\mu</math>l of lysate in assay) (2)</li> <li>5. CAT Enzyme Assay System with Reporter Lysis Buffer (up to 25<math>\mu</math>l of lysate in assay) (3)</li> </ol>	<ul style="list-style-type: none"> <li>• GenoTechnology NI™ Protein Assay</li> <li>• Pierce BCA Protein Assay with Compat-Able™ Protein Assay Preparation Reagent Set</li> <li>• Bio-Rad RC DC™ Protein Assay</li> <li>• Pierce BCA protein assay (dilute lysate 1:10)</li> <li>• Molecular Probes NanoOrange® Protein Quantitation Kit (dilute lysate 1:10)</li> </ul>	<ul style="list-style-type: none"> <li>• Compatible</li> <li>• Can release cytoplasmic, mitochondrial and nuclear contents (6)</li> </ul>
<b>Renilla Luciferase Assay Lysis Buffer (RLALB)</b>	<ol style="list-style-type: none"> <li>1. Renilla Luciferase Assay</li> <li>2. <math>\beta</math>-galactosidase assay (up to 45<math>\mu</math>l of lysate in assay)</li> <li>3. CAT Enzyme Assay System with Reporter Lysis Buffer (up to 25<math>\mu</math>l of lysate in assay)</li> </ol>	<ul style="list-style-type: none"> <li>• GenoTechnology NI™ Protein Assay</li> <li>• Pierce BCA Protein Assay with Compat-Able™ Protein Assay Preparation Reagent Set</li> <li>• Bio-Rad RC DC™ Protein Assay</li> <li>• Molecular Probes NanoOrange® Protein Quantitation Kit (dilute lysate 1:10)</li> </ul>	<ul style="list-style-type: none"> <li>• Not tested</li> </ul>

(1) Luciferase assays include all of our assays that are designed to measure only the activity of firefly luciferase and do not have extended signal half-life. These include products with catalog numbers E1500, E1501, E4030, E4530, E4550 and E1483.

(2) May be used in place of 1X RLB to lyse cells and dilute cell lysates in the  $\beta$ -Galactosidase Enzyme Assay, provided that a different stop buffer is used. We recommend the use of 1M Tris base as a stop buffer when samples are in 1X CCLR and 1X GLB because addition of the 1M Sodium Carbonate (provided with the system) causes a precipitate to form and absorbance readings are no longer accurate.

(3) Activity will not be as high as that detected in RLB. If CAT expression is very low, RLB may be necessary for optimal sensitivity.

(4) When performing a protein assay using any of the suggested protein assay systems, an equal concentration of lysis buffer must be used to generate the standard curve as well for optimal accuracy. If problems arise with the protein assay, further dilute the lysate with water and perform the protein assay on the macro, not micro, scale.

(5) Western blot analysis was performed using either AP- or HRP-conjugated secondary antibodies and colorimetric detection with TMB stabilized substrate for HRP or Western Blue® for AP. 1X, 2X and 5X concentrations of the Promega lysis buffers were tested.

(6) The ability of the lysis reagents to lyse different cellular organelles (cell membrane, nucleus, mitochondria) was investigated. This was done by probing the Western blots for a cytoplasmic antigen (ERK1/2), a mitochondrial antigen (cytochrome oxidase subunit I), or a nuclear antigen (histone). The efficiency of lysis may depend on growth conditions and cell or tissue type.