

Luciferase Assays

<u>Triton/Glycylglycine Lysis Buffer</u>	<u>Stock</u>	<u>Add to make 250 ml</u>
1% (vol/vol) Triton X-100	100%	2.5 ml
25 mM Glycylglycine (Gly-Gly, Sigma) pH 7.8	250 mM	25 ml
15 mM MgSO ₄	1 M	3.75 ml
4 mM EGTA	100 mM	10 ml
1 mM DTT added just before use		
Store at 4°C		H ₂ O to volume

<u>Luciferase Assay Buffer</u>	<u>Stock</u>	<u>Add to make 250 ml</u>
25 mM Glycylglycine pH 7.8	250 mM	25 ml
15 mM Potassium Phosphate pH 7.8	1 M	3.75 ml
15 mM MgSO ₄	1 M	3.75 ml
4 mM EGTA	100 mM	10 ml
2 mM ATP added just before use		
1 mM DTT added just before use		
Store at 4°C		H ₂ O to volume

<u>Luciferin Stock Solution</u>	<u>Stock</u>	<u>Per Sample</u>
1 mM D-luciferin, synthetic crystalline (Sigma)	5X*	40 µl
25 mM Glycylglycine	250 mM	20 µl
10 mM DTT	1 M	2 µl (just before use)
Store 1-ml aliquots in light-tight box at -80°C		140 µl H ₂ O

*5X Luciferin Stock: 5 mg luciferin, 1.8 ml 250 mM Glycylglycine, 180 µl 1 M DTT, 15.9 ml H₂O

PBS

Protocol:

- Place plates on ice. Wash plates 3x with ice cold PBS.
- Add 350 µl (for 6 cm plate) or 200 µl (for 3.5 cm plate) Triton/Glycylglycine Lysis Buffer

Per plate, mix:	<u>x1</u>	<u>x5</u>	<u>x15</u>
T/Gg buffer	350 µl	1750 µl	5250 µl
DTT (1 M stock)	0.35 µl	1.75 µl	5.25 µl

- Scrape with cell lifter; transfer to microcentrifuge tube; centrifuge for 5 min at 14,000 rpm at 4° C

- Place 100 μl of lysate in 2054 tube (Fisher); Add 360 μl luciferase assay buffer

Per sample, mix:	<u>x1</u>	<u>x5</u>	<u>x15</u>
Luc Assay buffer	360 μl	1800 μl	5400 μl
ATP (0.1 M stock)	7.2 μl	36 μl	108 μl
DTT (1 M stock)	0.36 μl	1.8 μl	5.4 μl

- Make Luciferin stock solution (need 200 μl /sample) or thaw frozen aliquot (recipe above); keep covered with foil
- Read luciferase activity with Autolumat luminometer