LUCIFERASE ASSAY

(David Dankort)

a) Assemble the following reagents

i) Bring 1.2x-Assay solution to room temperature You will require 80μl of buffer A per well plus an additional 800μl.

1x-Lysis Buffer ii) Mix 1 part 5x-DLUC lysis bufer with 4 parts ice cold ddH_2O and store on ice until use. You will require 150μl per 35mm plate.

b) Rinse plate twice with ice cold PBS.

c) To each 35 mm plate add 150μl of 1x-DLUC lysis buffer and incubate on ice for 15-25min, occasionally rocking the plates.

d) Thaw 5x-Substrate buffer on ice (in the dark).

e) Using a CoStar scraper, scrape the attached cells from the dish and transfer solution to a chilled eppendorf tube. Pellet debris by centrifugation at 4°C, 1300rpm for 2-5 min.

f) Transfer the supernatent to a new chilled microcentrifuge tube. At this point the extract can be stored indefinitely at -80C. Beware that luciferase does not tollerate repeated freeze thaws well.

g) Remove 10-20μl of extract to a Luminometer sample tubes (Sarstedt 5ml, 75mmx12mm, cat# 55.476.005) and allow contents to come to room temperature before preceeding.

h) Make the follwoing assay mix just prior to reading samples.

Assay Mix For each plate, make an **Assay Mix** by mixing 20μl of 5x-Substrate buffer (Buffer B) with 80μl of buffer A (1.2x-Assay Solution) add an additional 200μl of Buffer B and 800μl of buffer A. E.g. for 25 plates mix 700μl of buffer B and 2800μl of buffer A.

h) Inject 100μl of the assay mix into an extract sample and measure the light produced over 10 seconds following a 2 second intial delay to aloow compete mixing of reagents.

i) Quantitate the concentration of protein in each sample by mixing the same amount of extract (10-20μl) with 1ml of a 1 part BioRad Bradford Assay 4 part ddH_2O mixture. Calculate the amount of protein in each lysate using a freshly made standard curve.

REAGENTS REQUIRED [final]

5x-DLUC-Lysis Buffer

50ml	0.5M	Trizma Phosphate* [pH 7.75]	125mM
2ml	1M	DTT	10mM
100ml	100%	Glycerol	50%
10ml	100%	Triton X-100	5%
10ml	0.2M	EGTA	10mM

Bring to a total of 200ml with ddH_2O. Stored at -20°C until needed.

5x-Substrate Buffer (B)

5ml	2mg/ml	Luciferin*	2333μM
2ml	1M	Coenzyme A*	1333μM
0.4ml	100mM	ATP*	2666μM

Bring to a total of 15ml with ddH_2O, aliquot in 1.2ml aliquots and store in the dark at -80°C until needed. The luciferin is very UNSTABLE; it oxidizes quickly and breaks down in the light. Keep aliquots in the dark and wrap aliquots to be used in tin foil while thawing.

1.2x-Assay Solution (A)

10ml	250mM	glyclyglycine* [pH 7.75]	31.25mM
10ml	150mM	$MgSO_4$	18.75mM
200μl	1M	DTT	2.5mM
20μl	0.5M	EDTA	125μM
59.78ml		ddH_2O	0.1%

This can be stored at 4°C for a few months.

250mM	**glyclyglycine*** [pH 7.75]	3.3025g in 100ml pH to 7.75
150mM	**$MgSO_4$**	3.6114g in 200ml
0.5M	**Trizma Phosphate*** [pH 7.75]	43.82g/200ml pH to 7.75 with NaOH
2mg/ml	**Luciferin***	10mg Luciferin in 5ml of 25mM gylcyglycine pH7.75, 20mM $MgCl_2$ (cat # B.M.C. 411 400)
1M	**Coenzyme A***	50mg CoEnzyme A (lithium salt Grade I) in 6.51ml of 25mM gylcyglycine pH7.75, 20mM $MgCl_2$ (cat # B.M.C. 103 497)
100mM	**ATP***	1g ATP (disodium salt, special quality) in 16.5ml of ddH_2O (cat # B.M.C. 519 979)
