GUS Assays

I. Protein isolation

A. Method for ~1g or more of tissue.

1. Label all tubes. Prepare solutions and have ready at hand.

2. Remove the tissue from the –80^{o}C freezer and thaw on ice. If the tissue is fresh, keep on ice (or alternatively work in a cold room).

3. Place tissue in a mortar and pestle.

4. Add ~ 2ml of QB per ~1g tissue.

5. Grind tissue until no more chunks are visible.

6. Remove ~1ml of the liquid grindate into a microfuge tube.

7. Place on ice.

8. Rinse mortar and pestle (and any other paraphernalia that came into contact with the sample) to remove all traces of sample and proceed to the protein isolation of the next tissue sample.

9. Spin samples at top speed in the microfuge (4^{o}C for 15+ minutes).

10. Transfer the liquid supernatant into a second (new) microfuge tube.

11. Sometimes excess tissue is transferred over into the second microfuge tube. If this is the case, spin a second time for about 10 minutes and transfer this supernatant into a third microfuge tube.

12. Store samples in the –80^{o}C.

B. Alternative method for small (<1g) quantities of tissue.

1. Working in the fume hood, prepare a pestle by flaming the end of a blue pipette tip and sealing the end by gently smashing it into a microfuge tube. Prepare as many pestles as tissue samples to be isolated.

2. Using the newly created pestle, grind the tissue directly in a microfuge tube.

3. Add ~1ml of QB to the ground tissue, mix and transfer the supernatant to a second microfuge tube.

4. Follow the procedure in A above the rest of the way.

II. Gus Assays

A. Preparations

1. Remove the protein samples from the –80^{o}C freezer and thaw on ice.

2. Label all test tubes etc. in which assay will be performed.

3. Turn on and set circulating water bath to 37^{o}C.

B. The reaction

1. Aliquot 400μL GUS assay buffer to each test tube.

2. Place tubes into the 37^{o}C water bath.

3. Add 5μL of protein sample to the prewarmed test tube at 37^{o}C.

4. Note time.

5. Add next sample at a convenient time interval.

   (For example: add protein sample #1 to the first test tube containing GUS assay buffer and at 15 seconds add protein sample #2 to the second tube containing GUS assay buffer and at 30 seconds add protein sample #3 to the third tube containing GUS assay buffer etc until the entire set to be analyzed has been added to the GUS assay buffer containing tubes.)

6. Add 1.6ml Stop buffer to each tube after sufficient time has elapsed. * Add the Stop at the same time intervals at which the protein was added to the GUS assay buffer containing tubes.

   (For example if protein sample was added to the tubes containing GUS assay buffer at 0, 15, 30, 45, 60 ... sec, add Stop buffer at 15-min 0-sec, 15-min 15-sec, 15-min 30-sec, 15-min 45-sec, ... etc.)

   The time interval can be variable (the final result is expressed as a function of time: nnmol product released per minute per μg of protein) but should be linear over time; i.e., the relative fluorescence at 10 minutes should be 2X the relative fluorescence at 5 minutes. In order to accomplish this requirement it may be necessary to dilute the protein sample an order of magnitude or more

(i.e., 10X dilution, 100X dilution etc). Before beginning with the full-blown GUS assay of all samples perform a trial assay in order to determine that the assay is linear over time.

C. The assay

1. Turn on the TKO 100 fluorometer 15 minutes (or more) before use.

2. Calibrate and set the settings on the fluorometer.

(a) Fill the glass cuvette with 1.9 ml carbonate stop buffer.

(b) Place the cuvette into the sample chamber and close the lid. The "G" should be face forward.

(c) Adjust the scale knob all the way to clockwise.

(d) Adjust the "zero" knob until the display reads zero.

(e) Add 100µl of the 1µM MU standard solution to the cuvette containig the 1.9 ml carbonate stop buffer and mix (by inversion or up and down pipetting). Place the cuvette into the chamber and close the lid.

(f) Adjust the scale knob so that the TKO 100 reads 500. Thus a 50nM solution of MU will read 500 and the TKO 100 display; therefore 10 display counts per nM MU.

III. Bradford protein concentration determination assays

A. Measure the protein concentration in the extract using the dye-binding assay of Bradford (1976):

1. Dilute the Bradford reagent fivefold in dH_2O (1 part Bradford: 4 parts dH_2O). Filter the diluted reagent through Whatman 540 paper (or equivalent, I use the Millipore filtration unit).

2. Add 5-20 µl of the protein extract to 1 ml of the diluted reagent and mix. Measure the blue color formed at 595 nm. Use disposable plastic cuvettes to prevent the formation of a blue film.

Note: The Beckman DU 640 spec housed in Al Barta's lab is equipped with a sipper; thus the sample can be sucked right out of a microfuge tube directly into the spec eliminating the need to use cuvettes.

3. Prepare a standard curve using a serial dilution series (0.1-1.0 mg/ml) of a known protein sample concentration; e.g., BSA dissolved in QB.

4. Determine the protein concentration of the plant extract from the regression curve of the known sample.

IV. Express the results as nmol product released per minute per μg of protein.

$$\frac{\text{nM MUG hydrolyzed}}{\text{minute - μg protein}}$$

If the fluorometer was set such that 10 display counts equaled 1nM MU then 10 counts is equivalent to 1nM MUG hydrolyzed, 1000 counts is 100nM MUG hydrolyzed. Minutes is the duration of the GUS reaction time interval and μg protein is the quantitly of total protein that was used in the GUS assay; e.g., 5μL of 1mg per ml plant protein sample used in the GUS reaction would be 5μg of protein.

V. Solutions and stuff

A. Solutions

1. QB

Stock	For 100ml	Final []
2M KPO_4 (pH 7.8)	5ml	100mM
0.5M EDTA	200µL	1mM
Triton X-100	1ml	1%
80% Glycerol	12.5ml	10%
dH_2O	81.1ml	

Store RT

DTT (1.0M)	100µL	1mM

(Alternatively add 15.4mg DTT per 100ml)
Add DTT immediately before using. Store QB w/DTT at –20°C.

2. GUS assay buffer

Stock	For 25ml	Final []
QB	25ml	
DTT	50µL	
MUG	22mg	

(Methylumbelliferyl β-D-glucuronide)
Store –20°C.

3. MU calibration stock

(a) 1mM MU

(1) MU 9.9mg
(2) dH_2O to 50ml
(3) Store in brown bottle at 4°C.

(b) 1µM MU

(1) 1mM MU 10µL
(2) dH_2O 10ml
(3) Store in brown bottle at 4°C.

(c) 50nM MU calibration solution

(1)	1μM MU	100μL
(2)	Na_2CO_3 Stop	1.9ml
(3)	Make fresh immediately before each use.	

4. 0.2M Na_2CO_3 Carbonate Stop Buffer

Stock	For 1000ml	Final []
Na_2CO_3	21.2g	0.2M
dH_2O to	1000ml	

5. 2M KPO_4 (pH 7.8)

Stock	For 200ml	Final []
K_2HPO_4	63.2g	
KH_2PO_4	5.0g	
pH should be	~7.8	
If not, adjust pH to	7.8	
F, a/c		

6. 0.5M EDTA (pH 8.0)

Stock	For 250ml	Final []
EDTA	46.52g	0.5M
H_2O to	250ml	
pH w/ 10N NaOH to	8.0	
(Alternatively use ~5 pellets of NaOH.)		
f, a/c.		
Store RT.		

Note: EDTA will not completely go into solution until the pH approaches 8.0 and the H_2O is almost at final volume. Essentially, the pH needs to be continuously adjusted as the EDTA dissolves.

7. 10N NaOH

Stock	For 250ml	Final []

NaOH	100g	10N
dH_2O to	250ml	

Store at RT in a PLASTIC bottle. (NaOH will react with glass.)

8. 80% Glycerol

Stock	For 100ml	Final []
100% Glycerol	80ml	80%
dH_2O	20ml	
a/c		

9. 1M DTT

Stock	For 10ml	Final []
DTT	1.545g	1M
0.01M NaOAc (pH 5.2)	to 10ml	

Filter sterilize
Aliquot into 1ml portions
Store at –80°C

01M NaOAc is 33µL of 3M NaOAc pH~5.2 in 9.67ml dH_2O.)

B. Stuff

1. Mortar and pestle and/or flame seal blue tips.
2. Microfuge tubes, pipette tips.
3. Test Tubes
4. Timer

VI. References

Bradford, M.M. (1976) A dye binding assay for protein. *Anal. Biochem.* **72**:248-254.

Gallagher, S.R. (ed.) (1992) GUS protocols using the GUS gene as a reporter of gene expression. Academic Press, Inc.

Jefferson, R.A. (1987) Assaying chimeric genes in plants: the GUS gene fusion system. *Plant Mol. Biol. Rep.* **5**:387-405.

Jefferson, R.A. and Wilson, K. J. (1991) The GUS gene fusion system. Plant Molecular Biology Manual B14:1-33.

QB:

Ni, M., Dehesh, K., Tepperman, J.M., and Quail, P.H. (1996) GT-2: In vivo transcriptional activation activity and definition of novel twin DNA binding domains with reciprocal target sequence selectivity. *Plant Cell* **8**:1041-1059.

High Throughput

Perkin Elmer HTS 7000 Bioassay Reader
PE: 1/800/762-4000

Filters:

Excitation: 360nm (365nm desired:)
Emission: 465nm (455nm desired)

White polystyrene 96 well plates; Costar cat #392
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