GUS ASSAY

The Quasi Definitive Guide to Determining β-Glucuronidase Activity in Cell Lysates (special bonus protocols for the harvesting and lysis of E. coli, Rhodococcus and Corynebacterium)

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Adapted from Wilson *et al*., The *Escherichia coli gus* Operon: Induction and Expression of the *gus* Operon in *E.coli* and the Occurrence and Use of GUS in Other Bacteria. From **GUS Protocols: Using the GUS Gene as a Reporter of Gene Expression**. Ed. Sean R. Gallagher, Academic Press, Inc., 1992.

Harvesting of Cultures

4. At appropriate time points during the culture, remove 1.6 ml of culture into a 2ml Eppendorf tube and place on ice.
5. Working quickly, briefly vortex the 1.6 ml sample and remove 100 uL into a spectrophotometer cuvette containing 900 uL fresh culture media. Spin down the remaining 1.5 ml sample, remove the supernatant and place the pellet immediately at -80°C. (Note: Samples will keep at -80°C indefinitely; do NOT freeze at -20°C.)
6. Measure and note the OD_{600} of the 1:10 dilution. (Note: The OD_{600} of your sample will factor into the later calculation of GUS activity.)

Lysing of Cells

8. Thaw sample pellets on ice.
9. Resuspend pellets in 374 uL B-PER™ Bacterial Protein Extraction Reagent (Pierce product 78248), 50 uL Protease and Phosphatase Inhibitor Cocktail for use with bacterial cell extracts (Sigma product P8465; reconstituted per manufacturer's indications), 1 uL 34 mg/mL chloramphenicol (prepared in methanol), and 6 uL 10mg/ml lysozyme (prepared *fresh* in dH_20; can be omitted when working with *E. coli*).
10. Vortex vigorously for 1 min.
11. Incubate on ice for 5 min.
12. At this point, what you have is a crude lysate slurry that can be assayed for GUS activity using the procedure in the next section yielding a measure of activity in terms of nM product formed/minute $\cdot OD_{600}$ unit. (Note: For *Corynebacterium* and *Rhodococcus* this is the best you can hope for using this lysis procedure).
13. For *E. coli* samples, spin down the crude lysate 1 min and place on ice.
14. What you have now is a cleared lysate (the supernatant cleared of cell debris). The protein concentration of the cleared lysate can be determined using the Bradford assay (see the separate protocol for this assay). The cleared lysate can be assayed for GUS activity using the procedure described in the next section, yielding a measure of activity in terms of nM product formed/minute $\cdot$ mg protein. (Note: Activity per mg protein is a more desirable measure of activity than activity per OD_{600} unit. Think about it.)

GUS Assay

1. Keep cell lysates on ice.
2. Prepare *fresh* GUS assay buffer and prewarm to 37°C.
3. Prepare disposable cuvettes (at least 3 per assay), each containing 800 uL 0.4 M Na_2CO_3 (stop solution).
4. Prepare one 2 mL Eppendorf tube per sample containing 800 uL of the GUS assay buffer prepared as above. Add 200 uL cell lysate to the tube. (This gives a final concentration of PNPG 1mM.) Note time.
5. Place reactions at 37°C and note the time. (Note: A water bath is preferable, but consistency is key.) Watch for development of yellow color (*p*-nitrophenol). At no fewer than 3 time points, (e.g. 15, 30, 45 minutes) remove 100 uL from each sample into a spectrophotometer cuvette. (Note: When assaying crude lysate slurry, tap spin the reaction tubes and remove 100 uL of the supernatant into the spectrophotometer cuvette.) Briefly vortex the reaction tubes and return to 37°C quickly. The time points do not have to be equally spaced, but be sure to note each time.
6. Measure absorbance at 405 nm for each time point against a stopped blank reaction. (Note: The color formation is stable in the stop solution. Less error is introduced if you measure the absorbance of all of the time points at once rather than after each time point.)

Calculating the Rate of Reaction

1. For each sample, plot a graph of OD_{405} (y-axis) vs. time (x-axis). Calculate the slope S of the graph (which should be linear!) in OD_{405} units per minute. (Or cheat and have Excel calculate it for you)
2. The rate of reaction R (in nanomoles product per minute per OD_{600} unit)** for each sample is then:

$$R = S/(0.02 \times V \times OD_{600})$$

where V is the volume assayed in milliliters. In this case, V = 0.02 mL because 0.2 mL sample is used in the initial reaction (step 3) and one-tenth of this (100 uL) is removed into stop solution for each time point (step 4). The 0.02 in the denominator has been derived from the molar extinction coefficient of *p*-nitrophenol. Under the conditions specified by this particular protocol, the molar extinction coefficient of *p*-nitrophenol is 18,000. In the 900 uL final volume, an absorbance of 0.02 represents 1 nmol of product produced.

**The rate of reaction R can also be calculated in terms of nanomoles product per minute *per mg protein:*

$$R = S/(0.02 \times V \times \text{protein concentration})$$

The Bradford Assay can be used to determine the protein concentration of each sample.

Reagents

GUS Assay Buffer
[50 mM NaPO4, pH7, 1mM EDTA, 5mM DTT, 1.25mM PNPG]
for 50 mL:
49.13mL GUS Buffer Stock Solution
250 uL 1M DTT
625 uL 100mM PNPG
Prepare ***fresh*** and prewarm to 37°C

GUS Buffer Stock Solution
[50 mM $NaPO_4$, pH7, 1mM EDTA]
for 200 mL:
7.8 mL 0.5M NaH_2PO_4
12.2 mL 0.5M Na_2HPO_4
0.4 mL 0.5M EDTA, pH8
180mL distilled H_2O
Prepare ahead and store at room temperature.
DTT and PNPG need to be added before use.

0.5M NaH_2PO_4
for 500 mL:
34.5g NaH_2PO_4 anhydrous
dH20 to 500mL
Prepare ahead and store at room temperature.

0.5M Na_2HPO_4
for 500 mL:
35.5g Na_2HPO_4 anhydrous
dH20 to 500mL
Prepare ahead and store at room temperature.

0.5M ethylenediamine tetraacetic acid (EDTA)
for 200 mL:
dissolve 37.22g $Na_2EDTA \cdot 2H_2O$ in 140 mL dH2O
adjust pH to 8.0 with 10N NaOH (~10 mL)
dH_2O to 200 mL
Prepare ahead and store at room temperature.

1M dithiothreitol (DTT)
for 10 mL:
1.54g DTT
dH_2O to 10ml
Prepare ahead and store 1 mL aliquots at -20°C.

100mM *p*-nitrophenyl β-D-glucuronide (PNPG)
for 1 mL:
0.032g PNGP
GUS Buffer Stock Solution to 1 mL
Prepare ***fresh*** and keep on ice.

0.4 M Na_2CO_3 (Stop Solution)
for 500ml:
21.2g Na_2CO_3
dH_2O to 500ml
Prepare ahead and store at room temperature.

10mg/mL lysozyme
(if working with *Rhodococcus* or *Corynebacterium*)
for 1 mL:
10mg lysozyme
dH2O to 1mL
Prepare ***fresh*** and keep on ice.

B-PER™ Bacterial Protein Extraction Reagent
Pierce ready-to-use product 78148, 500mL

Protease and Phosphatase Inhibitor Cocktail for use with bacterial cell extracts
Sigma product P8465
Reconstituted per manufacturer's indications
Prepare ahead and store 200 uL aliquots at -80°C

34 mg/ml chloramphenical
for 10 mL
340mg chloramphenical
methanol to 10mL
Prepare ahead and store 1 mL aliquots at -20°C.