Microtiter Plate Beta-Galactosidase Assay Protocol

Adapted by Vinh Pham from Michael Goodson, Ph.D protocols.
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MATERIALS

Wash Buffer (10mM Tris pH 7.5, 5mM DTT)

Glass Beads (Ballotini Grade 8 <0.400-0.520mm>–Jencons Scientific cat#136-015)

To prepare: Soak in 1M HCl for 1hr, rinse by swirling twice in distilled water, and bake at 250ºC o/n.

Z Buffer (60mM Na_2HPO_4, 40mM NaH_2PO_4, 10mM KCl, 1mM $MgSO_4$, pH 7.0)

0.5M Chlorophenol Red- _-D-Galactopyranoside (CPRG, Roche Biochemicals cat#0884308, 250mg)

To prepare: Dissolve by adding 640□l dH_2O to 250mg CPRG in original vial.
Dilute 1-2□l CPRG in □fuge tubes with dH_2O to a [10^{-5}].
Measure A_{420}. The desired A_{420} range is 0.13 +/- 0.05. If needed, dilute CPRG in original vial with dH_2O to attain this range.
Recheck A_{420}.
Store in original vial at -20ºC

96-well Clear, Flat-Bottom Polystyrene Microtiter Plate (Costar cat#9017)

PROCEDURE

Part I – Sample Preparation

1. Grow yeast cells in 3-5ml of the appropriate Synthetic Complete (SC) medium (also known as Synthetic Dropout medium) to an OD_{600} reading of at least 0.5 (aim for a range of 0.7-1.3 for consistency). For yeast 2-hybrid strains, use SC trp^- leu^-.

   Note: As a positive control, use strain KSC647, which contains plasmids with SNF1-Gal4 AD and SNF4-Gal4 BD fusion constructs originally made by Stephen J. Elledge, Ph.D. This strain was obtained from the Kaplan lab.

2. Pellet at max speed for 1’.
3. Wash 3 times with 1ml Wash Buffer.
4. Resuspend pellet in 25-30□l Wash Buffer.
5. Add approximately 1 volume of acid-washed glass beads.

   Note: Add beads to weighing paper, then transfer desired amount to a □fuge tube.

6. Vortex on high for 1’.
7. Incubate on ice for 1’.
8. Repeat steps 6 & 7 four more times (for a total of 5 vortex/ice incubation steps).
9. Pellet at max speed for 5”.
10. Transfer s/n to a fresh □fuge tube.
11. Add 2.5□l 3M KCl.
12. Spin at max speed for 10’ at 4ºC.
13. Transfer s/n to a fresh □fuge tube.
14. Quick-freeze extracts with liquid nitrogen or a dry-ice ethanol bath.

15. Store at -20ºC or -80ºC.

Note: A freeze or no freeze experiment showed that freezing the extracts does not adversely affect _-galactosidase activity.

Part II – _-Galactosidase Assay

1. Set microplate reader temperature to 37ºC and allow to warm up for 10'.
2. Prepare fresh Assay Substrate.

Z Buffer	1000□l (5 samples)	6ml (30)	12ml (60)
0.5M CPRG	1.0□l	6□l	12□l
-Mercaptoethanol (-ME)	1.4□l	8.4□l	16.8□l

Note: Do NOT allow more than the desired volume of CPRG to thaw. Scrape off the required amount and allow to thaw on side of tube before pipetting.

3. (Optional) Warm Assay Substrate to 37ºC.
4. Add 20□l sample/well to a microtiter plate (Unknowns). Use 20□l of Wash Buffer as a negative control (Blank).
5. Load plate into reader (use "Drawer" button to open and close).
6. Launch SOFTmax® Pro application.
7. Under "Assays" menu, select "CPRG(B galactosidase Assay)."
8. Click "Setup" button and change the following readings, if needed:

Mode: Kinetic	Wavelength: 550nm	
Run Time: 1 hour	Automix	Before First Read: 5 secs
Interval: 1 minute		Between Reads: 3 secs

9. Click "Display" button, select "Plot" and check the "With reduced number" box.
10. Click "Template" button.
11. Select experimental sample fields and group as "Unknowns."
12. Place negative control field in "Blank" group.
13. Click OK to close template.
14. Click Read.
15. While the plate is being read, measure [sample protein] by Bradford Protein Assay using the remaining 5-10□l of sample.
16. After 1 hour have elapsed, export file as a .txt file to ZIP disk.
17. Open file in Microsoft Excel. The numbers at the bottom are the Vmax values for each sample (computed through a linear regression algorithm), which measure milli OD_{550} units per minute. Each Vmax value is the slope of a particular plot, and it is proportional to the amount of product formed per unit time.
18. Divide these readings by the [protein]'s from step 15 to get arbitrary _-galactosidase specific activity units (milli-OD_{550} per min. per mg of protein). Disregard samples with OD_{595} readings below 0.15.
19. Display results in bar graph.