Introduction

The gene encoding β-galactosidase (lacZ) of E.coli has been widely used as a reporter gene in many different prokaryotic and eukaryotic organisms. In particular, this gene has proven useful for studying gene expression in the yeast S. cerevisiae.

In addition to its utility in studying the regulation of gene expression, the measurement of β-galactosidase activity can be used to identify protein:protein interactions in vivo using two-hybrid systems. The strength of the interaction is usually verified and/or quantitated using a β-galactosidase activity assay.

In contrast to methods using 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside (X-gal) as a β-galactosidase substrate, our reagent system allows for the qualitative or quantitative determination of β-galactosidase activity in solution directly from colonies growing on solid medium. Part of a colony is picked from a plate and resuspended in a mixture of Y-PER™ Yeast Protein Extraction Reagent and β-galactosidase assay buffer. After a brief incubation period, the solution turns yellow from the hydrolysis of o-nitrophenyl-β-D-galactopyranoside (ONPG) to o-nitrophenol (ONP) and galactose in a mildly alkaline solution. The assay becomes quantitative if the quantity of cells in the assay is first determined with an absorbance reading taken at 660 nm (OD$_{660}$).

Common protocols for determining β-galactosidase activity from liquid cultures involve harvesting the cells by centrifugation, washing the cells several times, and lysing the cells by either subjecting them to freeze/thaw cycles or by treating them with detergents and/or organic solvents. These traditional protocols are not amenable to projects that involve screening cells growing in 96-well plates. Our system allows the researcher to assay cell cultures directly with no harvesting or washing steps. The cells used in this assay can be obtained from a large growing culture, cell culture plates or colonies growing on a solid medium. The working reagent is prepared by simply mixing equal volumes of the Y-PER™ Yeast Protein Extraction Reagent and the 2X β-galactosidase Assay Buffer. This reagent can be added directly to cells growing in culture.

Yeast Colony β-galactosidase Assay Protocol - Qualitative Method

1. Thaw the 2X β-galactosidase Assay Buffer on ice.
2. Once thawed, dilute a volume of this assay buffer with an equal volume of Y-PER™ Reagent to make the working solution. Each colony to be assayed will require 50 µl-100 µl of this working solution.
3. Pipet 50 µl-100 µl of the working solution into each microcentrifuge tube. Prepare one tube for each colony to be assayed.
4. Using a sterile inoculation loop, toothpick or pipette tip, transfer a portion of a single colony to one of the microcentrifuge tubes containing the working solution. Mix gently with a vortex mixer to create a homogeneous solution.

5. Incubate this reaction tube at room temperature or 37°C (optimal) until a color change is observed. **Note:** Hydrolysis of ONPG will lead to the production of a yellow color typically within minutes, but this is dependent on the amount of β-galactosidase present in the sample. **Note:** If the yellow color is not readily apparent, read the absorbance of the solution at 420 nm against a blank containing only the working solution. This will prove especially useful in samples with low levels of β-galactosidase activity.

**Yeast Colony β-galactosidase Assay Protocol - Quantitative Method**

1. Thaw 2X β-galactosidase assay buffer on ice. Each colony to be assayed will require 250 µl of the Y-PER™ Yeast Protein Extraction Reagent and 250 µl of the 2X β-galactosidase Assay Buffer.

2. Pipet 250 µl of Y-PER™ Reagent into a microcentrifuge. Prepare one tube for each colony to be assayed.

3. Using a sterile inoculation loop, toothpick or pipette tip, transfer a portion of a single colony to one of the microcentrifuge tubes containing the Y-PER™ Reagent. Mix gently with a vortex mixer to create a homogeneous solution.

4. Determine the OD 660 of the solution and record this value.

5. Add 250 µl of 2X β-galactosidase Assay Buffer to the microcentrifuge tube and start the timer.

6. Incubate this reaction tube at room temperature or 37°C (optimal) until a color change is observed. **Note:** Hydrolysis of ONPG will lead to the production of a yellow color typically within minutes, but this is dependent on the amount of β-galactosidase present in the sample.

7. When yellow color appears, add 200 µl (i.e., 0.4 volumes) of β-Galactosidase Assay Stop Solution to the reaction tube and vortex for 15 seconds. Stop the timer and record the total reaction time.

8. Remove the cell debris from the reaction tube by centrifugation at 13,000 x g for 30 seconds.

9. Transfer the supernatant to a cuvette and read the absorbance of the solution at 420 nm against a blank tube containing 250 µl of Y-PER™ Reagent, 250 µl of the 2X β-galactosidase Assay Buffer, and 200 µl of the β-Galactosidase Assay Stop Solution. **Note:** The reaction time will vary depending on the level of β-galactosidase expression in the test colony. To be within the linear range of the assay, the A 420 should be between 0.02-1.0.

10. Determine the β-galactosidase activity in the test reaction using the following equation: β-galactosidase activity = 1,000 x A 420/(t x V x OD 660), where t= time (in minutes) of incubation and V = volume of cells (ml) used in the assay (0.25 in the above example). A comparison of the β-galactosidase activity between test samples will allow you to determine the relative difference in the activity of this enzyme between colonies (ΔA 420/min x ml x OD 660 of culture).

**Yeast β-galactosidase Microwell Plate Assay Protocol (nonstopped)**

1. Thaw 2X β-galactosidase Assay Buffer on ice.

2. Once thawed, dilute a volume of this assay buffer with an equal volume of Y-PER™ Reagent to make the working solution. Each 96-well microwell plate will require approximately 10 ml of the working solution (100 µl/well). The maximum culture volume allowed per well with this protocol is 100 µl.

3. Determine the OD 660 of all test cultures and record their values. Be sure to include one well in the assay (see Step 5) that contains only the growth media (no cells) to serve as a blank for the microplate spectrophotometer.

4. Add 100 µl of each culture to individual wells in the 96-well microwell plate. Noting the time at which the working reagent is added (using a timer), apply 100 µl of the working reagent to each well.

5. Incubate the plate at room temperature for approximately 30 minutes. Determine A 420 of each well in the 96-well plate, using the well containing the medium alone (no cells) to zero the microplate spectrophotometer. **Note:** The reaction
time will vary depending on the level of β-galactosidase expression in the different test cultures. To be within the linear range of the assay, the A420 should be between 0.02-1.0.

6. Determine the β-galactosidase activity in the test reactions using the following equation: β-galactosidase activity = 1,000 x A420/(t x V x OD660), where t = time (in minutes) of incubation and V = volume of cells (ml) used in the assay (0.1 in the above example). A comparison of the β-galactosidase activity between test samples will allow you to determine the relative difference in the activity of this enzyme between cultures (ΔA420/min x ml x OD660 of culture).

Yeast β-galactosidase Microwell Plate Assay Protocol (stopped)

1. Thaw 2X β-galactosidase Assay Buffer on ice.
2. Once thawed, dilute a volume of this assay buffer with an equal volume of Y-PER™ Reagent to make the working solution. Each 96-well microwell plate will require approximately 7 mL of the working solution (70 µl/well). The maximum culture volume allowed per well with this protocol is 70 µl.
3. Determine the OD660 of all test cultures and record their values. Be sure to include one well in the assay (see Step 7) that contains only the growth media (no cells) to serve as a blank for the microplate spectrophotometer.
4. Add 70 µl of each culture to individual wells in the 96-well microwell plate. Noting the time at which the working reagent is added (using a timer), apply 70 µl of the working reagent to each well.
5. When yellow color appears, add 56 µl (i.e., 0.4 volumes) of 1M Na2CO3 stop solution to each well and mix gently for 15 seconds. Stop the timer and record the total reaction time.
7. Determine A420 of each well in the 96-well plate, using the well containing the medium alone (no cells) to zero the microplate spectrophotometer. **Note:** The reaction time will vary depending on the level of β-galactosidase expression in the different test cultures. To be within the linear range of the assay, the A420 should be between 0.02-1.0.
8. Determine the β-galactosidase activity in the test reactions using the following equation: β-galactosidase activity = 1,000 x A420/(t x V x OD660), where t = time (in minutes) of incubation and V = volume of cells (ml) used in the assay (0.07 in the above example). A comparison of the β-galactosidase activity between test samples will allow you to determine the relative difference in the activity of this enzyme between cultures (ΔA420/min x ml x OD660 of culture).

Microcentrifuge Tube Protocol

1. Grow cell cultures to mid-log phase (OD660 of 0.5-1.0). Record the exact OD660 of each culture.
2. Thaw 2X β-galactosidase Assay Buffer on ice. Once thawed, dilute a volume of this assay buffer with an equal volume of the Y-PER™ Reagent to make the working solution. Each assay will require approximately 350 µl of the working solution.
3. Set up a blank tube containing 350 µl of culture medium (no cells), 350 µl of the working solution and 300 µl of the 1M Na2CO3 stop solution.
4. Transfer 350 µl of each test culture to a microcentrifuge tube.
5. Noting the time at which the working reagent is added (using a timer), apply 350 µl of the working reagent to each test culture.
6. Incubate this reaction tube at room temperature or 37°C (optimal) until a color change is observed. **Note:** Hydrolysis of ONPG will lead to the production of a yellow color typically within minutes, but this is dependent on the amount of β-galactosidase present in the sample.
7. When yellow color appears, add 300 µl (i.e., 0.43 volumes) of β-Galactosidase Assay Stop Solution to the reaction tube and vortex for 15 seconds. Stop the timer and record the total reaction time.
8. Remove the cell debris from the reaction tube by centrifugation at 13,000 x g for 30 seconds.
9. Transfer the supernatant to a cuvette and read the absorbance of the solution at 420 nm against the blank tube prepared in Step 3. **Note:** The reaction time will vary depending on the level of β-galactosidase expression in the test culture. To be within the linear range of the assay, the $A_{420}$ should be between 0.02-1.0.

10. Determine the β-galactosidase activity in the test reaction using the following equation:

$$\text{β-galactosidase activity} = \frac{1,000 \times A_{420}}{t \times V \times OD_{660}}$$

where $t$ = time (in minutes) of incubation and $V$ = volume of cells (ml) used in the assay (0.35 in the above example). A comparison of the β-galactosidase activity between test samples will allow you to determine the relative difference in the activity of this enzyme between cultures ($\Delta A_{420}/\text{min x ml x OD}_{660}$ of culture).