INSTRUCTIONS

Yeast β-Galactosidase Assay Kit

75768

0856.3

Number	Description
75768	**Yeast β-Galactosidase Assay Kit**
	Kit Contents:
	Y-PER™ Yeast Protein Extraction Reagent, 25mL, store at room temperature
	2X β-Galactosidase Assay Buffer, 25mL, store at -20°C
	β-Galactosidase Assay Stop Solution, 25mL, contains 1 M Na_2CO_3, store at room temperature
	Storage: Upon receipt store individual components as indicated. Product is shipped with dry ice.

Introduction

The Thermo Scientific Yeast β-Galactosidase Assay Kit allows for qualitative or quantitative determination of β-galactosidase activity in solution directly from colonies growing on solid medium. A portion of the colony is suspended in a mixture of Thermo Scientific 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 cell quantity is first determined by measuring the optical density at 660nm.

The gene encoding β-galactosidase (*lacZ*) of *E. coli* has been widely used as a reporter gene in many different prokaryotic and eukaryotic organisms. β-Galactosidase activity is also commonly used as an indicator of protein:protein interactions *in vivo* using two-hybrid systems. The interaction strength is verified or quantitated using a β-galactosidase activity assay.

Common protocols for determining β-galactosidase activity from liquid cultures involve harvesting cells by centrifugation, washing cells several times, and lysing cells by either subjecting them to freeze/thaw cycles or by treating them with detergents or organic solvents. These traditional protocols are not amenable to projects that involve screening cells growing in 96-well plates. The Yeast β-Galactosidase Assay Kit allows cells obtained from solid media or liquid culture to be assayed directly with no harvesting or washing steps.

Yeast Colony β-galactosidase Assay Protocol - Qualitative Method

1. Thaw the 2X β-Galactosidase Assay Buffer on ice.
2. Add a volume of the assay buffer to an equal volume of Y-PER Reagent to make the working solution (WS). Each colony requires 50-100µL of the WS.
3. Pipette 50-100µL of the WS into each microcentrifuge tube. Prepare one tube for each colony to be assayed.
4. Use a sterile inoculation loop, toothpick or pipette tip to transfer a portion of a single colony to one of the microcentrifuge tubes containing the WS. Mix gently with a vortex mixer to create a homogeneous solution.
5. Incubate reaction tube at room temperature or 37°C (optimal) until a color change is observed.

   Note: The solution will become yellow within minutes depending on the amount of β-galactosidase in the sample. If the color change is not apparent, measure the absorbance at 420nm against a blank containing the WS, which is useful for 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.
2. Pipette 250µL of Y-PER Reagent into a microcentrifuge tube. Prepare one tube for each colony.
3. Use a sterile inoculation loop, toothpick or pipette tip to transfer a portion of the colony to one of the tubes containing the Y-PER Reagent. Mix gently with a vortex mixer to create a homogeneous solution.

   Note: For calculations, use 0.25mL for cell volume.
4. Determine the OD_{660} of the solution and record this value.
5. Use a timer to monitor the reaction. Add 250µL of 2X β-Galactosidase Assay Buffer to the microcentrifuge tube and start timer.
6. Incubate the reaction tube at room temperature or 37°C (optimal) until a color change is observed.

   Note: The solution will become yellow within minutes depending on the amount of β-galactosidase in the sample.
7. When the color change appears, add 200µL 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 centrifuging at 13,000 × *g* for 30 seconds.
9. Transfer supernatant to a cuvette and measure the absorbance at 420nm against a blank containing 250µL of Y-PER Reagent, 250µL of the 2X β-Galactosidase Assay Buffer, and 200µL of the β-Galactosidase Assay Stop Solution.

   Note: The reaction time will vary depending on the level of β-galactosidase expression. Absorbance values between 0.02-1.0 are within the linear range of the assay.
10. Proceed to Calculations section.

Yeast β-galactosidase Microplate Plate Assay Protocol (non-stopped)

1. Thaw 2X β-Galactosidase Assay Buffer on ice.
2. Add a volume of this assay buffer with an equal volume of Y-PER Reagent to make the working solution (WS). Each well requires 100µL of the WS. The maximum culture volume allowed per well is 100µL.
3. Determine the OD_{660} of all test cultures and record values. Include one well that contains growth media only (no cells) as a blank for the spectrophotometer.
4. Add 100µL of each culture to the wells.
5. Use a timer to monitor the reaction. Apply 100µL of the WS to each well and start timer.
6. Incubate the plate at room temperature for approximately 30 minutes.
7. Use the well containing medium only (no cells) to zero the instrument. Measure the absorbance at 420nm of each well.

   Note: The reaction time will vary depending on the level of β-galactosidase expression. Absorbance values between 0.02-1.0 are within the linear range of the assay.
8. Proceed to Calculations Section.

Yeast β-galactosidase Microplate Plate Assay Protocol (stopped)

1. Thaw 2X β-Galactosidase Assay Buffer on ice.
2. Add a volume of the assay buffer to an equal volume of Y-PER Reagent to make the working solution (WS). Each well requires 70µL of the WS. The maximum cell culture volume allowed per well is 70µL.
3. Determine the OD_{660} of all test cultures and record values. Include one well that contains only the growth medium (no cells) as a blank for the instrument.
4. Add 70µL of each culture to individual wells in the microplate.
5. Use a timer to monitor the reaction. Apply 70µL of the WS to each well and start the timer.

6. When the yellow color appears, add 56µL of β-Galactosidase Assay Stop Solution to each well and mix for 15 seconds. Stop the timer and record the total reaction time.
7. Use the well containing medium only (no cells) to zero the instrument. Measure the absorbance at 420nm of each well.

   Note: The reaction time will vary depending on the level of β-galactosidase expression in the different test cultures. Absorbance values between 0.02-1.0 are within the linear range of the assay.
8. Proceed to Calculations Section.

Microcentrifuge Tube Protocol

1. Grow cell cultures to mid-log phase (OD_{660} of 0.5-1.0). Record the exact OD_{660} of each culture.
2. Thaw 2X β-Galactosidase Assay Buffer on ice.
3. Add a volume of assay buffer to an equal volume of the Y-PER Reagent to make the working solution (WS). Each assay will require 350µL of the WS.
4. Prepare a blank tube containing 350µL of culture medium (no cells), 350µL of the WS and 300µL of the β-Galactosidase Assay Stop Solution.
5. Transfer 350µL of each test culture to a microcentrifuge tube.
6. Use a timer to monitor the reaction. Apply 350µL of the working reagent to each test culture and start timer.
7. Incubate the reaction tube at room temperature or 37°C (optimal) until a color change is observed.

   Note: The solution will become yellow within minutes depending on the amount of β-galactosidase in the sample.
8. When the yellow color appears, add 300µL of β-Galactosidase Assay Stop Solution to the reaction tube and vortex for 15 seconds. Stop the timer and record the total reaction time.
9. Remove cell debris from the reaction tube by centrifuging at 13,000 × *g* for 30 seconds.
10. Transfer supernatant to a cuvette and measure the absorbance at 420nm against the blank tube.

    Note: The reaction time will vary depending on the level of β-galactosidase expression in the test culture. Absorbance values between 0.02-1.0 are within the linear range of the assay.

Calculations

To measure β-galactosidase activity, use the following equation:

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

t= time (in minutes) of incubation
V = volume of cells (ml) used in the assay

Related Thermo Scientific Products

78990	**Y-PER Yeast Protein Extraction Reagent,** 500mL
75706	**β-Galactosidase Assay Stop Solution,** 25mL
75707	**Mammalian β-Galactosidase Assay Kit**

Product Reference

1. Moriya, H. and Johnston, M. (2004). Glucose sensing and signaling in Saccharomyces cerevisiae through the Rgt2 glucose sensor and casein kinase I. PNAS. **101(6):**1572-7.

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