

# Mammalian $\beta$ -Galactosidase Assay Kit

75707 75710

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Number	Description
75707	<b>Mammalian <math>\beta</math>-Galactosidase Assay Kit</b> <b>Kit Contents:</b> <b>Pierce™ <math>\beta</math>-Galactosidase Assay Reagent</b> , 25mL, (Product No. 75705) <b>M-PER™ Mammalian Protein Extraction Reagent</b> , 25mL (may be purchased separately as Product No. 78503, see Related Thermo Scientific Products) <b><math>\beta</math>-Galactosidase Assay Stop Solution</b> , 25mL, (Product No. 75706)
75710	<b>Pierce <math>\beta</math>-Galactosidase Assay Reagent</b> , 3 × 25mL (3 × Product No. 75705)

**Storage:** Upon receipt store the Pierce  $\beta$ -Gal Assay Reagent at -20°C. Store all other components at room temperature. Product shipped on dry ice.

## Table of Contents

Introduction .....	1
Procedure for Adherent Cells in a Microplate .....	1
Procedure Cells in Suspension.....	2
Procedure for Cell Lysates.....	3
Related Thermo Scientific Products .....	3

## Introduction

The Thermo Scientific Mammalian  $\beta$ -Galactosidase Assay Reagent Kit is a simple and versatile assay to measure  $\beta$ -galactosidase activity in cultured mammalian cells. Cells grown on a plate may be lysed and tested for  $\beta$ -galactosidase activity by adding a single solution. Alternatively, Thermo Scientific M-PER Mammalian Protein Extraction Reagent may be used to prepare lysate from washed adherent or pelleted cells, and then a portion of the lysate used to assay for  $\beta$ -galactosidase activity. With M-PER Reagent, there is no need to scrape cells or to perform freeze-thaw cycles. The remaining lysates prepared with M-PER Reagent may be used for applications such as reporter assays (luciferase,  $\beta$ -galactosidase and chloramphenicol acetyltransferase), protein kinase assays (PKA, PKC, and tyrosine kinase), immunoassays (Western blot, ELISA, RIA) and protein purification.

## Procedure for Adherent Cells in a Microplate

Two methods to measure  $\beta$ -Galactosidase activity from adherent cells are presented. Method 1 is a procedure to assay total cell lysate in a well. Method 2 is a procedure to lyse and measure only a portion of the cell lysate, reserving some of the lysate for other purposes.

- **Method 1: Directly assay all cells**

1. Slowly thaw and equilibrate the  $\beta$ -Galactosidase Assay Reagent to room temperature (do not heat).
2. Wash cells once with phosphate buffered saline (PBS), pH 7.2.
3. Add 100 $\mu$ L of  $\beta$ -Galactosidase Assay Reagent to each well. (If a portion of the lysate is to be used for another assay, see Method 2).
4. Cover plate and incubate for 30 minutes at 37°C.

5. Stop reaction by adding 100-150 $\mu$ L of  $\beta$ -Galactosidase Assay Stop Solution to each well.  
**Note:** Stopping the reactions is optional. However, if the absorbance is to be measured using a plate reader immediately after incubation, it is not necessary to stop the reactions.
6. Measure absorbance at 405nm.

- **Method 2: Assay a portion of the lysate**

1. Wash cells once with phosphate buffered saline (PBS), pH 7.2.
2. Add 50 $\mu$ L M-PER Reagent to each well.
3. Incubate plate at room temperature for 5 minutes with gentle shaking.
4. Remove the portion of lysate to be reserved for other purposes.
5. Add an amount of  $\beta$ -Galactosidase Assay Reagent equal to the volume of lysate remaining in the well.
6. Cover plate and incubate for 30 minutes at 37°C.
7. Stop the reaction by adding 100-150 $\mu$ L of  $\beta$ -Galactosidase Assay Stop Solution to each well.  
**Note:** Stopping the reactions is optional. However, if the absorbance is to be measured using a plate reader immediately after incubation, it is not necessary to stop the reactions.
8. Measure absorbance at 405nm.

## Procedure Cells in Suspension

1. Slowly thaw and equilibrate the  $\beta$ -Galactosidase Assay Reagent to room temperature (do not heat).
2. Harvest cells by centrifugation at 2500  $\times$  g for 10 minutes. Discard supernatant.  
**Note:** If using adherent cells, wash and trypsinize cells before performing step 2.
3. Wash cells by re-suspending the pellet in a suitable volume of a desired wash buffer (e.g., phosphate buffered saline, PBS). Centrifuge cells again at 2500  $\times$  g for 10 minutes. Discard supernatant.
4. Add M-PER Reagent to the cell pellet. For every 1 gram of wet cell paste, add 10mL of M-PER Reagent (see Table 1 for M-PER Reagent volume guidelines).  
**Note:** If a large amount of cell paste is used, add 1/10 the final volume of M-PER Reagent to cell pellet. Gently agitate or pipette mixture to re-suspend cells until a homogeneous solution is obtained, then add the remainder of the required M-PER Reagent to the cell suspension.
5. Gently agitate cell suspension for 10 minutes at room temperature.
6. Remove cell debris from lysate by centrifugation at 27,000  $\times$  g for 15 minutes. The supernatant obtained from this treatment is clarified cell extract.
7. Transfer 50 $\mu$ L of the clarified cell extract into a microplate well.
8. Add 50 $\mu$ L of the  $\beta$ -Galactosidase Assay Reagent to the well.
9. Cover plate and incubate for 30 minutes at 37°C.
10. Stop the reaction by adding 100-150 $\mu$ L of  $\beta$ -Galactosidase Assay Stop Solution to each well.  
**Note:** Stopping the reactions is optional. However, if the absorbance is to be measured using a plate reader immediately after incubation, it is not necessary to stop the reactions.
11. Measure absorbance at 405nm.

## Procedure for Cell Lysates

1. Slowly thaw and equilibrate the  $\beta$ -Galactosidase Assay Reagent to room temperature (do not heat).
2. Transfer 50 $\mu$ L of cell extract into a microplate well.
3. Add 50 $\mu$ L of the  $\beta$ -Galactosidase Assay Reagent to the well.
4. Incubate for 30 minutes at 37°C.
5. Stop the reaction by adding 100-150 $\mu$ L of  $\beta$ -Galactosidase Assay Stop Solution to each well.  
**Note:** Stopping the reactions is optional. However, if the absorbance is to be measured using a plate reader immediately after incubation, it is not necessary to stop the reactions.
6. Measure absorbance at 405nm.

**Table 1. Suggested volume of Thermo Scientific M-PER Reagent for different-sized plates.**

Plate Diameter	Volume of M-PER Reagent
100mm	500-1000 $\mu$ L
60mm	250-500 $\mu$ L
6-well plate	200-400 $\mu$ L per well
24-well plate	100-200 $\mu$ L per well
96-well plate	50-100 $\mu$ L per well

## Related Thermo Scientific Products

<b>75705</b>	<b>Pierce <math>\beta</math>-Galactosidase Assay Reagent, 25mL</b>
<b>75706</b>	<b><math>\beta</math>-Galactosidase Assay Stop Solution, 25mL</b>
<b>78503</b>	<b>M-PER Mammalian Protein Extraction Reagent, 25mL</b>
<b>78501</b>	<b>M-PER Mammalian Protein Extraction Reagent, 250mL</b>
<b>78243</b>	<b>B-PER™ Bacterial Protein Extraction Reagent, 165mL</b>
<b>78248</b>	<b>B-PER Bacterial Protein Extraction Reagent, 500mL</b>
<b>75768</b>	<b>Yeast <math>\beta</math>-Galactosidase Assay Kit</b>
<b>78990</b>	<b>Y-PER™ Yeast Protein Extraction Reagent, 500mL</b>
<b>23225</b>	<b>Pierce BCA Protein Assay Kit</b>
<b>23236</b>	<b>Pierce Coomassie Plus (Bradford) Protein Assay Kit</b>

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