

lac Operon

An operon is a unit of gene expression and a transcriptionally-regulated system. The *lac* operon is responsible for producing the proteins that control the uptake of lactose for the use as a carbon energy source when glucose is not available to the cell. It consists of three structural genes and a repressor gene. The enzymes required for lactose utilization coded for by the operon are as follows: β -galactosidase, lactose permease, and thiogalactoside transacetylase. β -galactosidase hydrolyzes the bond between glucose and galactose (the two sugars that constitute the disaccharide, lactose) and is coded for by the gene *lacZ*. Lactose permease acts to bring lactose into the cell from the outside; the cell is otherwise impermeable to the sugar. It is coded for by the gene *lacY*. Thiogalactoside transacetylase has a cellular detoxification function and is coded for by the gene *lacA*. The repressor protein (Lac I) prevents expression of the above genes.

IPTG

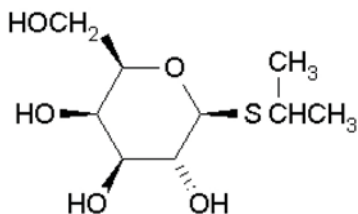
IPTG (Isopropyl β -D-thiogalactopyranoside) is a galactose analog that acts as an inducer of the *lac* operon. IPTG binds to the repressor protein that would normally turn off the *lac* operon the same way galactose would. Without the repressor protein binding to the operator site, expression of the *lac* operon or other, engineered lac-inducible genes ensues.

Cloning procedures that require the induction of β -galactosidase activity often use IPTG.

IPTG is soluble in water at 250 mg/mL.

Cat. No. 46-102-RF
 Size 1 x 1 g
 Storage: -5 to -20°C
 Shipping: -5 to -20°C

Formula: C₉H₁₈O₅S
 Molecular Weight: 238.3 g/mol



Blue/White Screening

Included in agar:

Add 5 mL X-gal stock solution (20 mg/mL) and 5 mL 0.1 M IPTG per 1 L autoclaved media agar (cooled to 55°C or below). Add the appropriate antibiotics and pour into plates. Plate cells on agar and incubate overnight at 37°C.

Application to top of agar:

Add 40 μ L of X-gal stock solution (20 mg/mL) and 4 μ L of a 200 mg/mL IPTG solution to the top of agar plates. Spread solution evenly over the agar and incubate at 37°C until the fluid is not visible on the agar surface. Plate cells and incubate overnight at 37°C.

X-Gal

X-Gal (5-Bromo-4-chloro-3-indolyl β -D-galactopyranoside) is a substrate of β -galactosidase. In the presence of β -galactosidase, X-gal is cleaved to yield indoxyl, an insoluble blue precipitate. X-gal is often used in cloning procedures that require detection of a foreign DNA inserted into the region of the *lac* operon encoding β -galactosidase. Insertion of the DNA into the *lacZ* gene results in a loss of β -galactosidase activity and, therefore, no production of insoluble blue precipitate in the presence of X-gal. As a result, cells containing the inserted DNA by color when plated on media containing X-gal. This technique is often referred to as blue/white screening.

X-Gal solutions are prepared in dimethylsulfoxide (DMSO) at 20 mg/mL.

Cat. No. 46-101-RF
 Size 1 x 1 g
 Storage: -5 to -20°C
 Shipping: -5 to -20°C

Formula: C₁₄H₁₅BrClNO₆
 Molecular Weight: 408.6 g/mol

