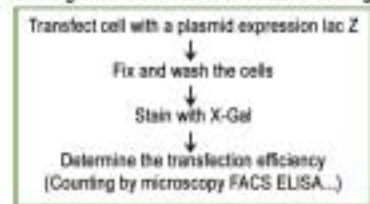


$\beta$ -Galactosidase, encoded by E.coli lacZ gene, is one of the most common reporter gene marker used in molecular biology applications : it is commonly used for monitoring transfection efficiency in mammalian, yeast, and bacterial cells and identifying expression of recombinant fusion genes<sup>1-4</sup>. E. coli  $\beta$ -galactosidase has a high turnover rate, hydrolyzing D-galactose from various labeled  $\beta$ -galactosides. Since this enzyme is generally absent in normal mammalian, yeast, some bacterial and even plant cells, it can be detected at very low levels, and due to its wide substrate specificity, monitoring lacZ expression (and therefore co-expressed genes or promoter efficiency) has become routine to the point of detection of as few as 5 copies of  $\beta$ -galactosidase.

### Staining scheme in Blue/White selection cloning



### Literature :

- 1- J Appl Bacteriol 64, 65 (1988)
- 2- Appl Environ Microbiol 35, 136 (1978)
- 3- Zentralbl Bakteriol 280, 476 (1994)
- 4- Infect Immun 61, 5231 (1993)
- 5- Mol Cell Probes 9, 145 (1995)

Chromogenic assays of  $\beta$ -galactosidase activity (i.e. X-Gal) are very popular, being the standard dye for blue/white selection in cloning. Now, Interchim also offers fluorogenic and chemiluminescent substrates for improved performances and new applications. Beside high quality chemicals, 3 staining kits are proposed.  $\beta$ -Galactosidase substrates can also be used in conjunction with galactosidase-conjugated secondary detection agents in enzyme linked immunosorbent assays (ELISA, FLISA) and immunohistochemical techniques.<sup>5</sup>

### Selection guide

Name	Color of released product (Fluor. Abs/Em in nm)	Cat.#
<b>Substrates and kits for <math>\beta</math>-Galactosidases</b>		
• <b>Chromogenic substrates</b>		
x-gal	Blue/Red/Rose/Green	page D.73 UP40534
Red- $\beta$ -D-Gal	Red	A2702
Rose- $\beta$ -D-Gal	Rose	AM341
Purple- $\beta$ -D-Gal	Purple	AM339
Green- $\beta$ -D-Gal	Green	AM338
o-NPG	Yellow	UP55668
• <b>Chromogenic kits</b>		
lacZ $\beta$ -Galactosidase Detection Kit		page D.72 J29660
• <b>Fluorogenic substrates</b>		
FDG	Green (490/514)	page D.73 FP-52476
FM-Gal	Green (490/514)	FP-52477
Res-Gal	Red (571/585)	FP-52473
MU-Gal	Blue (360/449)	FP-24874
TFMU-Gal	Aqua (385/501)	FP-M1141
CUG	Light Blue (330/448)	FP-M1171
• <b>Fluorogenic kits</b>		
FACS Blue lacZ $\beta$ -Galactosidase Detection Kit	Light Blue (330/448)	page D.72 FP-BM841
In vivo lacZ $\beta$ -Galactosidase Intracellular Detection Kit	Green(490/514)	M02590
Chemiluminescent substrates and kits		
Chemiluminescent lacZ $\beta$ -Galactosidase Detection Kit		BM8420
<b>Substrates and kits for <math>\beta</math>-Glucuronidases</b>		
• <b>Chromogenic substrates</b>		
X-GLUC	Indigo/Red/Rose	page D.74 UP194285
MUGlcU	Blue (360/450)	UP775560
<b>Substrates and kits for <math>\beta</math>-Glucosidases</b>		
• <b>Chromogenic substrates</b>		
X-GLU	Indigo	page D.75 UP193325

Interchim provides also substrates for  $\beta$ -Glucuronidase and  $\beta$ -Glucosidases, and other reporter enzymes on inquire.

### Lac Z $\beta$ -Galactosidase inducer (IPTG)

IPTG enhances  $\beta$ -galactosidase activity by binding and inhibiting the lac repressor. IPTG and a chromogenic substrate X-Gal are often used to identify bacterial colonies containing recombinant plasmid DNA for vectors containing the lacZ gene.

Description	Cat.#	Qty
IPTG	UP84853C	1 g
IPTG	UP84853D	10 x 1 g

# Cloning

## Enzyme Substrates and Kits for $\beta$ -Galactosidase

Contains sufficient reagents for stainings 100 assays in 35 mm wells (Cell Fixative Reagent, Cell Staining Solution and X-GAL Reagent).

### $\beta$ -Galactosidase Staining Kit, Colorimetric blue staining

The  $\beta$ -Galactosidase Histochemical Staining Kit is an efficient and simple method for assaying  $\beta$ -galactosidase reporter gene expression in situ in for in vitro and in vivo applications. Using this kit, transfection efficiency can be determined easily and reliably. The kit can be used following transient or stable transfections as well as in tissue sections following in vivo gene delivery. This colorimetric assay allows for direct visualization of individual cells expressing the reporter gene. The  $\beta$ -Galactosidase Histochemical Staining Kit provides all the reagents required for fast, accurate  $\beta$ -galactosidase transfection efficiency determination.

Description	Cat.#	Qty
$\beta$ -galactosidase Staining Kit	J29660	1 kit

### FACS Blue lacZ $\beta$ -Galactosidase Detection Kit, Fluorogenic blue staining

The FACS lacZ  $\beta$ -Galactosidase Detection Kit allows highly sensitive detection of  $\beta$ -galactosidase activity in mammalian, yeast, bacterial, or plant cells, at a shorter wavelength (blue fluorescence) than other kits. This kit includes CUG fluorogenic substrate that is several orders of magnitude more sensitive than X-Gal, and more water soluble. As a result, this assay is especially amenable to FACS, automated ELISA assays, and for co-staining with a green (fluorescein) labeled antibody or other assays in dual labeling experiments.

Description	Cat.#	Qty
FACS Blue lacZ $\beta$ -Galactosidase Detection Kit	FP-BM8410	10 x 96 tests

### In vivo lacZ $\beta$ -Galactosidase Intracellular Detection Kit

The in vivo lacZ  $\beta$ -Galactosidase Intracellular Detection Kit allows green fluorescent highly sensitive detection of  $\beta$ -galactosidase activity in live cells. It uses the fluorogenic substrate fluorescein di- $\beta$ -D-galactopyranoside (FDG) combined with Fluorescence microscopic analysis (confocal microscopy) analysis, that has been shown to be several orders of magnitude more sensitive than X-Gal substrate. It sensitively distinguishes lacZ+ vs. lacZ- cells. The kit is also useful in automated ELISA type assays.

Description	Cat.#	Qty
In vivo lacZ $\beta$ -Galactosidase Intracellular Detection Kit	FP-BN7260	1 kit

### lacZ $\beta$ -Galactosidase Detection Kit - Chemiluminescent

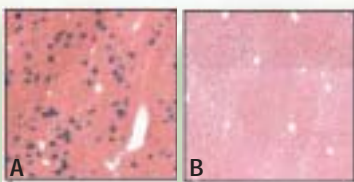
To measure galactosidase enzyme activity in mammalian, yeast, and bacterial cells

One of the most common reporter genes used in molecular biology applications is the *E. coli lacZ* gene that codes for an active subunit of  $\beta$ -galactosidase *in vivo*. Since this enzyme is generally absent in normal mammalian, yeast, some bacterial and even plant cells, it can be detected at very low levels, and due to its wide substrate specificity, monitoring *lacZ* expression (and therefore co-expressed genes or promoter efficiency) has become routine to the point of detection of as few as 5 copies of  $\beta$ -galactosidase. Although chromogenic assays of  $\beta$ -galactosidase activity (i.e. X-Gal) are useful, the recent application of chemiluminescent 1,2 Dioxetane substrates, which emit visible light upon enzyme catalysis, provide rapid results with very low background and high intensity signal. An enhancing solution is also provided with this kit to increase light production efficiency in solution-based assays by drawing water away from the signal production site. The Chemiluminescent lacZ  $\beta$ -Galactosidase Detection Kit provides all the necessary reagents, buffers, substrate, and protocols for sensitive and quantitative lacZ  $\beta$ -galactosidase activity assay.

Description	Cat.#	Qty
lacZ $\beta$ -Galactosidase Detection Kit - Chemiluminescent	BM8420	1 kit

Contains :  
0.5 ml substrate solution (4x)  
Reference standard  
1 ml inhibitor

Contains :  
Fluorescent Substrate  
Reference standard  
Inhibitors (chloroquine, PETG)



Strong Liver Expression of  $\beta$ -galactosidase from the pLIVE<sup>TM</sup>-lacZ Vector after Hydrodynamic Tail Vein Injection. The pLIVE<sup>TM</sup>-lacZ Vector (Panel A) was delivered to an ICR mouse using the hydrodynamic tail vein injection procedure and the TransIT<sup>®</sup>-QR Hydrodynamic Delivery Solution (BM4530). At 24 hours post-injection, the liver was harvested, sectioned and stained with X-gal using the Beta-Gal Staining Kit (J29660) to demonstrate  $\beta$ -galactosidase activity (blue cells). The cells were then counterstained with hematoxylin and eosin Y to stain the nuclei and cytoplasm, respectively. The control mouse (*lacZ* negative) in Panel B was stained in parallel to Panel A and contains no detectable  $\beta$ -galactosidase activity.

### Fluorogenic Substrates for $\beta$ -galactosidase

Fluorescein di- $\beta$ -D-Galactopyranoside (FDG)	Green (490/514)	FP-52476A	5 mg
Fluorescein mono- $\beta$ -D-Galactopyranoside (FM-Gal)	Green (490/514)	FP-524771	5 mg
Resorufin- $\beta$ -D-Galactopyranoside (Res-Gal)	Red (571/585)	FP-52473A	50 mg*
4-Methylumbellifery- $\beta$ -D-Galactopyranoside (MU-Gal)	Blue (360/449)	FP-248742	5 g*
4-Trifluoromethylumbellifery- $\beta$ -D-Galactopyranoside (TFMU-Gal)	Aqua (385/501)	FP-M1141A	25 mg
Carboxyumbellifery- $\beta$ -D-Galactopyranoside (CUG)	Light Blue (330/448)	FP-M1171A	10 mg
3-Carboxyumbellifery- $\beta$ -D-Galactopyranoside-BSA conjugate	Light Blue (330/448)	FP-BM8390	10 mg
$\beta$ -Galactosidase Sample Kit	Red (571/585), Green (490/514) Aqua (385/501)Blue (330/448)	FP-BM8400	1 kit

#### MUGalactopyranoside

##### 4-MethylUmbellifery- $\beta$ -D-Galactopyranoside

$C_{16}H_{18}O_8$  MW : 338.31

Substrate for fluorogenic assay of  $\beta$ -D-galactosidase

$\lambda_{abs}$  : 316 nm (\*360 nm)

$\lambda_{em}$  : 376 nm (\*449 nm)

Note\* : Absorbance / Emission of product 4-methylumbelliferone after enzyme hydrolysis

Description	Cat.#	Qty
MU galactopyranoside	UP248742	1 g

### Chromogenic Substrates for $\beta$ -galactosidase

Also available, are X-Gal derivatives with different colors :

	MW	Color ( $I_{max}$ )	Cat.#	Qty
Green- $\beta$ -D-Gal	309.3	green	AM338A	25 mg
Rose- $\beta$ -D-Gal	329.74	pink (~540 nm)	AM341A	100 mg
Red- $\beta$ -D-Gal	408.6	red/magenta (~565 nm)	A27020	100 mg
Purple- $\beta$ -D-Gal	421.19	purple (~575 nm)	AM339A	25 mg

The red and rose precipitates are easier to detect against the background of plant cells

#### X-Gal

**X-gal** is a chromogenic substrate for  $\beta$ -galactosidase used in conjunction with IPTG to identify bacterial colonies that contain recombinant plasmids by blue/white selection. X-gal forms an intense blue precipitate in the presence of  $\beta$ -galactosidase activity. In experiments using vectors containing the lacZ gene, recombinant colonies (white) can be identified quite readily from bacterial colonies that contain non-recombinant plasmids (blue).

Description	Cat.#	Qty
X-Gal	UP40534M	1 g
X-gal	UP40534N	5 x 1 g

#### o-NPG (o-Nitrophenyl- $\beta$ -D-galactopyranoside)

oNPG is a chromogenic substrate for  $\alpha$ -galactosidase that is enzymatically converted to a soluble yellow product. The yellow product is read at 410 nm making oNPG useful as a substrate for ELISA based assays.

Description	Cat.#	Qty
oNPG	UP556683	5 g

Related product:

Description	Cat.#	Qty
IPTG	UP84853C	1 g

Advantages :

- ◆ I Gain of time compared with other detection methods on E.coli (24 hours, direct method in plate) (7.9.10)
- ◆ Less than 1% false positive, less than 5% of false negative (7.8.9)
- ◆ overcome limitations encountered with chemiluminescent methods (11)

Literature

- 1.Ellis 1993
- 2.Bommineni 1993
- 3.Martin 1990
- 4.Restaino 1990
- 5.Watkins 1988
- 6.Delisle 1989
- 7.Gaudet 1996
- 8.Ceibin 1995
- 9.Sartory 1992
- 10.Frampton 1988
- 11.Van Pouckld 1997

# Cloning

## Enzyme Substrates and Kits for $\beta$ -Galactosidase

### Substrate of $\beta$ -glucuronidase

Substrate of  $\beta$ -glucuronidase giving insoluble and blue color.

The chromogenic substrate X-GLUC is used in a variety of applications for the detection of the  $\alpha$ -glucuronidase enzyme. It produces upon cleavage a localized color [intense indigo-blue chromophore (615 nm)], but several derivatives are available for other colors (see side note).

#### Advantages:

- Gain of time compared with other detection methods on *E.coli* (24hours, direct method in plate) (7.9.10)
- Less than 1% false positive, less than 5% of false negative (7.8.9)
- overcome limitations encountered with chemiluminescent methods (11)

#### Applications :

- **detection of *E. coli*** : X-GLUC has been used for the detection of  $\beta$ -glucuronidase in a variety of cells : it has reported applications in the detection of *E. coli* contaminatin in water (7.8.9.11), in samples food such as meat, dairy products, and shellfish (4.5). This chemical has clinical applications in the assessment of urinary tract infection by detecting the presence of *E. coli* (6).

- **gene reporting** : X-GLUC is extremely useful in identifying gene presence within most cell types, through the rapid detection of the GUS (*E. coli*  $\beta$ -glucuronidase) gene fusion marker, especially in plants. Applications include gene expression in transcription, translation, and protein transfer studies (2.3).

X-GLUC is available as CHA salt, and as the Na sat that has better water solubility than the latter. Both are Biotechnology grade.

#### Literature

- 1.Ellis 1993 ; 2.Bommineni 1993 ; 3.Martin 1990 ; 4.Restaino 1990 ; 5.Watkins 1988 ; 6.Delisle 1989 ; 7.Gaudet 1996 ; 8.Ceibin 1995 ; 9.Sartory 1992 ; 10.Frampton 1988 ; 11.Van Pouckld 1997

#### X-GLUC

5-Bromo-4-Chloro-3-Indolyl- $\beta$ -D-Glucuronic acid, cyclohexylamonium salt  
Biotechnology grade

$C_{20}H_{26}BrClN_2O_7$  ; MW 521.8

Substrate for  $\beta$ -glucuronidase that produces insoluble, intense indigo-blue chromophore (615 nm) after enzymatic hydrolysis.

Description	Cat.#	Qty
X-GLUCcha	UP194285	100 mg

#### X-GLUC Na

5-Bromo-4-Chloro-3-Indolyl- $\beta$ -D-Glucuronic acid, sodium salt

$C_{17}H_{12}BrClNO_7Na$  ; MW : 444.6

Biotechnology grade

Description	Cat.#	Qty
X-GLUC Na	UP775560	100 mg

Also available, are X-GLU derivatives with different colors :

	MW	Color ( $I_{max}$ )	Cat.#	Qty
Red- $\beta$ -D-GlcU,CHA	521.8	red (-565nm)	F19510	25 mg
Rose- $\beta$ -D-GlcU,CHA	421.0	rose (-540nm)	F19520	25 mg
Chromogenic $\beta$ -Glucuronidase Substrate Sampler Kit contains 5 mg each of X-GlcU CHA, Red- $\beta$ -D-GlcU CHA, and Rose- $\beta$ -D-GlcU CHA.			BP3390	1 kit

The red and rose precipitates are easier to detect against the background of plant cells

#### MUGlcU

4-MethylUmbelliferyl- $\beta$ -D-Glucuronic Acid

$C_{16}H_{16}O_9$  ; MW : 352.3

Biotechnology grade

Substrate of  $\beta$ -glucuronidase

Fluorogenic  $\beta$ -glucuronidase substrate that releases the blue fluorescent 4-methyl-7-hydroxycoumarin ( $\lambda_{ex}$   $\lambda_{em}$  : 360\450 nm) on enzymatic hydrolysis. The substrate is commonly used for identifying *E. coli* contamination and for detecting marker GUS gene expression in plants with high sensitivity.

Description	Cat.#	Qty
MUGlcU	UP37744A	100 mg

Substrate of  $\beta$ -glucosidase

## X-GLU

5-Bromo-4-Chloro-3-Indolyl- $\beta$ -D-GlucopyranosideC<sub>14</sub>H<sub>15</sub>BrClNO<sub>6</sub>; MW : 408.6

Biotechnology grade

This substrate of  $\beta$ -glucosidase gives an insoluble and blue color.

Description	Cat.#	Qty
X-GLU	UP193325	100 mg

Product Name	MW - [CAS]	Cat.#	Comments
Dexamethasone 21-O- $\beta$ -D-Galactopyranoside	554.62 [319426-57-8]	BU7711, 1mg	Useful inducer of gene expression, Abs: 239nm, Em : none - Soluble : DMSO, H <sub>2</sub> O
Chloramphenicol 1-O- $\beta$ -D-Galactopyranoside	485.28 [191476-32-1]	BU7721, 2mg	Upon enzymatic cleavage, chloramphenicol, an antibiotic, is produced, Abs : lmax = 278nm ; Em : noneSoluble - : DMSO, H <sub>2</sub> O
5-Fluorouridine- 5'-O- $\beta$ -D-Galactopyranoside	424.33 [149965-92-4]	BU7751, 5mg	Useful for lacZ specific release of 5-FUR, an anti- metabolic, Abs : 268nm ; Em : noneTetracycline
10-O- $\beta$ -D-Galactopyranoside	604.62 [319426-63-6]	BU7761, 2mg	Useful antibiotic in lacZ transfections, Abs : 268nm(e=18K), 355nm(13K); Soluble : MeOH, H <sub>2</sub> O(s), DMSO
1,2-di-O-octanoyl-3-O- $\beta$ -D- Galactopyranosyl <i>rac</i> -glycerol	506.64	BU7771, 5mg	Synthetic diglyceride analog that activates protein kinase C. Soluble : H <sub>2</sub> O, DMSO
1-Oleoyl-2-acetyl-3- $\beta$ -D- Galactopyranosyl <i>sn</i> -glycerol	560.71	BU7781, 5mg	Synthetic diglyceride analog that activates protein kinase C. Soluble : H <sub>2</sub> O, DMSO
Pyridoxine Galactoside	331.33	BU7791, 10mg	Vitamin B <sub>6</sub> analog (pyridoxine). Soluble : DMSO, H <sub>2</sub> O
Pyridoxal Galactoside	315.28	BU7801, 2mg	Vitamin B <sub>6</sub> analog (pyridoxine), useful media compnent for <i>lacZ</i> positive cell selection Soluble : DMSO, abs. EtOH
Myo-inositol Galactoside	342.30	BU7811, 5mg	Derivative of myo-inositol, a component of membrane phospholipidsSoluble : H <sub>2</sub> O, DMSO
Riboflavin Galactoside	538.50	BU7831, 2mg	Vitamin B <sub>2</sub> analog (riboflavin), for transfected <i>lacZ</i> cell selectionSoluble : H <sub>2</sub> O, DMSO, DMF
Pantothenic acid 2,4- di-O- $\beta$ -D-Galactopyranoside	543.53	BU7841, 10mg	Vitamin B <sub>5</sub> analog (pantothenic acid), for transfected <i>lacZ</i> cell selection. Soluble : DMSO, H <sub>2</sub> O
Thiamine Galactoside	786.50	BU7861, 2mg	Vitamin B <sub>1</sub> analog (thiamine), for transfected <i>lacZ</i> cell selection. Soluble: DMSO, H <sub>2</sub> O
Bromoxnilyl glucuronic acid triacetate methyl ester	593.19	BU7871, 2mg	Herbicide derivative, useful for selective release in GUS-positive plant systems. Soluble : DMSO, DMF, EtOH
Bromoxnilyl glucuronic acid methyl ester	467.08	BU7891, 2mg	Herbicide derivative, useful for selective release in GUS-positive plant systems
7-N-Benzoyl-Cephalosporanic acid L-glutamate ester	477.5	BU7921, 5mg	Cephalosporin C conjugate, released upon ampicillinase activity, Abs: 280nm

## Applications

All the galactoside derivatives will release the conjugated moiety upon action of  $\beta$ -galactosidase, and can be used thus as controlled bioactive or marker groups for lacZ transfected cell lines. Please ask for information.

In example, myo-inositol (released by Myo-inositol galactoside), is a precursor of inositol phosphates that act as second messengers in vivo, and a key component of membrane phospholipids, glycerophosphatidylinositol that anchors bind glycoproteins to cell membranes.

This derivative can also be used as a tissue culture media component that will be selectively released in lacZ transfected cell lines.

## Literature :

Want, W.T., et al., Anal. Biochem. 188:432 (1990).  
Ferguson, M.A.J., Williams, A.F., Ann. Rev. Biochem. 57:285 (1988).