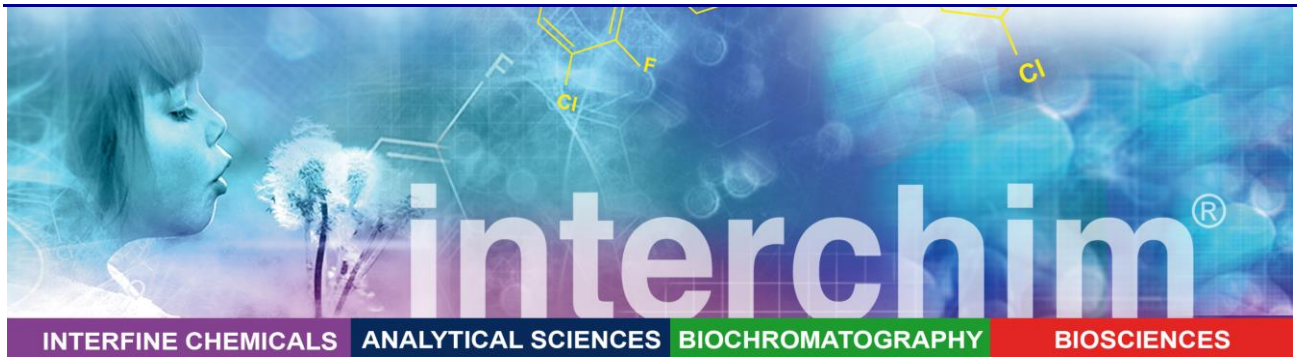
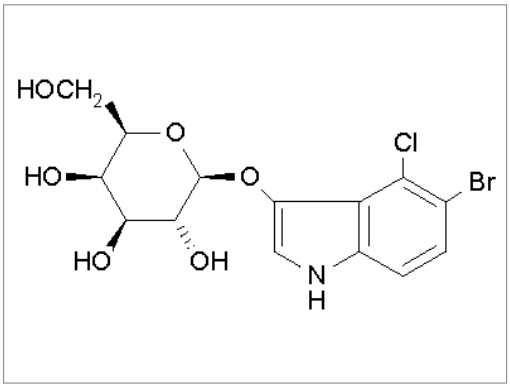


X-Gal and other Galatosidase substrates

Substrates for the detection of β-galactosidase (i.e. for lac Z gene recombinant system-based reporter assays, Lift colony, Histology localization, Blotting,…)

Products Description



Name:	**X-GAL** 5-Bromo-4-Chloro-3-Indolyl-b-D-Galactopyranoside		
Catalog #:	UP40534M, 1g UP40534P, 5 g 40534Q, 10 g	UP40534N, 10x1g	
Description	CAS: 7240-90-6 MW: 408.61 Purity **:** ≥ 99% Biotechnology grade		
Name & Catalog #:	**Blue-GAL** MW: 374.2	UP775580, 1g	Produces an insoluble blue-violet precipitate.
Name & Catalog #:	**Green-GAL** MW: 309.3	AM338A, 25mg	Produces an insoluble green precipitate.
Name & Catalog #:	**Rose-GAL** MW: 329.74	AM341A, 100mg	Produces an insoluble pink (ca 540nm) precipitate
Name & Catalog #:	**Red-GAL (Magenta-Gal)** MW: 408.6	A27020, 100mg	Produces an insoluble red/magenta (ca 565nm) precipitat
Name & Catalog #:	**Purple-GAL** MW: 421.2	AM339A, 100mg	Produces an insoluble purple (ca 575nm) precipitate.
Name & Catalog #:	**Green Japaleno-GAL** MW: 472.1	A2XK10, 0.2g	Produces an insoluble green precipitate.
Name & Catalog #:	**Bronze-GAL** MW: 443.47	A2XK20, 0.2g	Produces an insoluble bronze precipitate
Name & Catalog #:	**Brown Espresso-GAL** MW: 495.6	A2XK30, 0.2g	Produces an insoluble brown precipitate.
Name & Catalog #:	**Red Crimson-GAL** MW: 360.96	A2XK40, 0.2g	Produces an insoluble red precipitate.

Storage: dry at –20°C in dark (J)

See below more technical information on each compound

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Hotline +33 4 70 03 73 06 • interbiotech@interchim.com

Directions for use

X-Gal is widely used and you will find numerous protocols in the literature.

Protocol 1: X-gal detection of β-galactosidase reporter gene in colony dish cultures

1. Make tubes with 2.5 ml LB and 0.8 agar by melting then dispensing it into the tubes and autoclaving it for only 5 min. Use the tubes while hot or re-melt briefly and hold at 42 C.
2. Add 20 µl 20 mg/ml IPTG (filter sterilized in H_2O), 50 µl 20 mg/ml X-gal (in Dimethylformide), and antibiotic solution (i.e. 1 µl of Crb at 100 mg/ml).
   Note: Dimethylformide may melt plastic so make the stock in glass or PP (or PA) tubes.
3. Add the transformed cells (try to get about 200 CFU in up to 250 µl). Be sure to run controls.
4. Vortex and overlay on a CA plate containing the appropriate antibiotic (usually Crb).
   Note: 2.5 ml is a little tricky to overlay neatly: do not replace cap, tilt the plate to get uniform coverage of the overlay.
5. Let the agar solidify then incubate at 37 C until the blue color develops.
6. Pick the colourless colonies to media with the correct antibiotic and verify the insert by mini plasmid preparations or colony hybridization.

Protocol 2: X-gal Staining Protocol for slides

This protocol is modified from Dichek Lab, Gladstone/UCSF (r).

Preparation

1. Prepare Solution A: 5mM Potassium Ferricyanide Crystalline, 5mM Potassium Ferricyanide Trihydrate, 2mM Magnesium Chloride in 1 X PBS. Store at 4 deg, protected from light
2. Prepare X-gal Stock Solution (40X): 40 mg/ml in DMSO (100 mg in 2.5 ml DMSO). Store at -20 deg, protected from light

Staining procedure

1. Prepare final working X-gal Solution: dilute X-gal stock solution 1:40 in Solution A. First warm Solution A to 37 deg to prevent precipitation of X-gal.
2. Cut 10 micrometers cryostat sections onto pap-penned slides (Superfrost/ Plus Microscope Slides #U49860) from fresh-frozen tissues. Immerse immediately into cold formaldehyde/glutaraldehyde (2% Formaldehyde 0.2% Glutaraldehyde in 1 X PBS) 5 minutes, then rinse in dH20 for 60 seconds.
3. Let section dry completely onto slide Rinse with 1X PBS
4. Apply Final X-gal Solution to sections and incubate at 37 deg; for 30 minutes to 24 hours. Check sections under microscope every 2 hours for development of blue staining
5. Rinse with 1X PBS. Wash 2 X 2' with deionized H2O
6. Counterstain 3' with Nuclear Fast Red (also called Kernechtrot)
7. Wash as in washing step, then cover slip with Gel Mount

Protocol 3: β-galactosidase filter assay (lift colony)

1. Start with a plate of yeast colonies to be tested (often the result of the transformation of a library or a round of mutagenesis). Note: These colonies should contain the lacZ gene under the control of a promoter (i.e. FUS1).
2. For each plate to be tested, place a circle of 3MM Whatman paper (#BP2761] into each of two empty petri dishes. Note: The Whatman paper should cover the entire bottom of the petri dishes.
3. Add 2.5 ml of a-factor to the Whatman paper at the bottom of one of the petri dishes. If there are any bubbles, smooth them out with forceps. Note: Use a concentration of a-factor that produces a different response in wild-type versus mutant strains. This must be determined empirically.
4. Label a circular piece of nitrocellulose (NC) filter the size of the petri dish with a ball point pen.
5. Pick up the NC filter with tweezers and place it carefully across the plate of yeast colonies so that there are no bubbles or wrinkles in the filter. Let sit for 1-2 min.

6. While the NC filter is sitting on the yeast colonies, dip a needle in india ink and poke holes through the filter and into the solid media. This enables positive colonies on the filter to be matched with the colonies on the plate from which they were lifted.
7. Transfer the NC filter to a petri dish containing a-factor, colony side up.
8. Cover the dish and seal with parafilm. Put at 30° for 2.5 hrs.
9. During the 2.5 hr incubation, prepare and aliquot 2.5 ml of Z buffer + X-gal into the remaining petri dishes containing Whatman paper.
10. Z buffer + X-gal: 60 mM Na2HPO4 , 40 mM NaH2PO4oH2O , 10 mM KCl , 1 mM MgSO4o7H2O, 39 mM 2-mercaptoethanol , 1 mg/ml X-gal ; Adjust pH to 7.0.

Note: Z buffer without X-gal and without 2-mercaptoethanol can be stored at room temperature.
Note: X-gal should be stored as a 100 mg/ml stock in dimethylformamide (DMF) at -20°C.

11. Remove the plates from 30°, lift the NC filter off the Whatman paper, and place on an aluminium foil "boat." Then, slowly lower into an ice bucket containing liquid nitrogen.

Note: Freezing the cells in this way serves to permeabilize the membrane and allow the subsequent entry of X-gal, the substrate for b-galactosidase.
Note: The aluminium foil boat avoids having to manoeuvre the NC filter with tweezers. (The NC gets very brittle when frozen.)

12. After about 10 sec, carefully raise the boat to remove the NC filter from the liquid nitrogen.
13. Allow the NC filter to warm to room temperature (about 1 min).
14. Place the NC filter, colony side up, in the petri dish containing Z Buffer + X-gal.
15. Cover the dish and seal with parafilm. Put at 30° overnight.

Note: Blue color may start to appear after a few hours.

Protocol 4: X-gal staining for IHC and blotting with b-galactosidase conjugates

Perfom your ImmunoHistological slide (or blot) as usual. Saturation may be made with goat serum.

1. Shake off buffer and wipe off excess surrounding sections.
2. Completely cover tissues with X-GAL substrate at 1.22 mmol/L with potassium ferro/ferricyanide (each at 3 mmol/L).
3. Incubate for 15 - 60 minutes at room temperature or 37°C.
4. Rinse slides thoroughly in reagent quality water.
5. Dehydrate by rinsing briefly (10 dips) in 80% alcohol, 100% alcohol and xylene or xylene substitutes.
6. Mount in xylene-based mounting media, or use aqueous mounting media if tissues are thoroughly air dried.

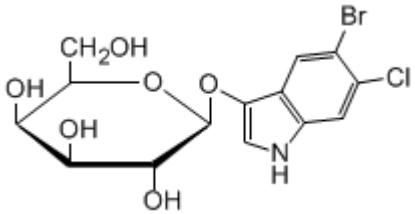
Tips for detection techniques

Note: paraffin embedding destroys ß-gal activity so there is no point in staining paraffin sections [F].

Reduction of the insoluble substrate by β-galactosidase forms a smaller moiety for electronic microscopic

Technical information

X-GAL



X-Gal (5-bromo-4-chloro-3-indolyl-b-D-galactopyranoside) is soluble in DMSO (>1mM), DMF
X-gal turns blue when incubated in the presence of β-galactosidase
X-gal is used a chromogenic substrate for β-galactosidase reporter gene, that is used on several of the cloning plasmids (especially, on the pUC series and lGT11 vectors, under the control of a promoter (i.e. FUS1)). When an inserted piece of DNA is placed in the correct restriction site, the lacZ gene is interrupted and the colony does not turn the media blue (desired colony appears white).

References X-gal

Sambrook 1989: Sambrook, J., Fritsch, EF, and Maniatis, T., in Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, NY, Vol. 1, 2, 3 (1989). I thought every laboratory has a copy of this.

Alternatives to X-Gal producing different colors for specific applications

Following are chromogenic substrates for β-D-galactosidase that yield upon cleavage a precipitate with different colors.

Blue-GAL (UP77558) is reported to produce a deeper blue water insoluble coloration than X-Gal.

Rose-GAL (AM341) and Red-Gal (A2702) are reported to be easier to visualize than X-Gal against the background of plant cells.

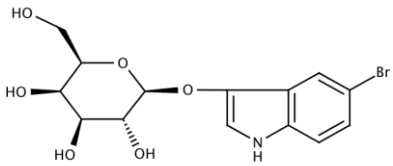
Magenta-Gal (A27020) is hydrolyzed by β-galactosidase to produce a magenta/red precipitate. This precipitate is insoluble in alcohol and xylenes, thus making it suitable for immunoblotting and immunocytochemical assays. Bacterial colonies containing active β-galactosidase produce red colonies when grown on plates containing Magenta-Gal. Magenta-Gal is commonly used in conjunction with IPTG for red/white screening.

Japaleno-GAL (A2XK10), Bronze-Gal (A2XK20), Espresso-Gal (A2XK30), and Crimson-Gal (A2XK40) are new color analogs, used at 0.5mM in petri dishes.

Blue-GAL (UP775580) Produces an insoluble blue precipitate.

Syn. : Blue gal; Bluo-gal; Blue-gal; 5-Bromo-3-indolyl β-D-galactopyranoside;
5-Bromo-3-indoxyl beta-D-galactopyranoside
CAS: 97753-82-7]

MW: 374.2

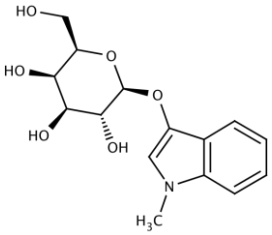


Purity : ≥ 99%
OD (465nm; 2% in DMF) < 0.01
Biotechnology grade
Produces an insoluble blue-violet precipitate.

Green-GAL (AM338A) Produces an insoluble green precipitate.

Syn.: N-Methyl-3-indolyl-b-D-galactopyranoside monohydrate
CAS: 207598-26-3

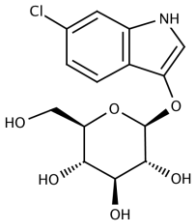
MW: 309.3



Rose-GAL (AM341A) Produces an insoluble pink (ca 540nm) precipitate

Syn. : 6-Chloro-3-indolyl b-D-galactopyranoside, Rose-Gal; Salmon Gal; Rose Gal;
6-Chloro-3-(b-D-galactopyranosyloxy)indole; 6-Chloro-3-indolyl β-D-galactopyranoside
CAS: 138182-21-5

MW: 329.74



Off-white to pale pink powder

Specific Optical Rotation : -40.0 - -50.0 ° (c=1, MeOH)
Water (KF) : ≤ 1.0%
Purity (HPLC) ≥ 99.0%

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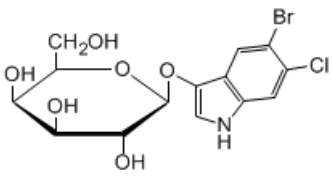
Magenta/Red-GAL (A27020) Produces an insoluble red/magenta (ca 565nm) precipitate.

Syn. : 5-Bromo-6-chloro-3-indoxyl β-D- galactopyranoside
MagentaTM-β-D-galactoside; MagentaTM-β-D-Gal, Red-Gal
CAS [93863-88-8]*

MW: 408.63 g/mol



Purple-GAL **Produces an insoluble purple (ca 575nm) precipitate.**
Syn. : 5-Iodo-3-indoxyl-β-D-galactopyranoside ; Iodo-β-D-Gal AM339A, 100mg
CAS : 36473-36-6
MW: 421.19g/mol
Off-white crystalline powder

Jalapeno - GAL **Produces an insoluble green precipitate.**
Syn. : Jalapeño green-Gal Jalapeno green-beta-D-galactoside; Jalapeno green–beta-D-galactopyranoside A2XK10, 0.2g
MW: 472.1 g/mol

Bronze - GAL **Produces an insoluble yellow/bronze precipitate.**
Syn. : ; Bronze-beta-D-galactoside; Bronze-beta-D-galactopyranoside A2XK20, 0.2g
MW: 443.57 g/mol

Espresso -GAL **Produces an insoluble brown precipitate.**
Syn. : Espresso-beta-D-galactoside; Espresso brown-beta-D-galactopyranoside A2XK30, 0.2g
MW: 495.6 g/mol

Crimson -GAL **Produces an insoluble red precipitate.**
Syn. : Crimson-beta-D-galactoside; Crimson red–beta-D-galactopyranoside A2XK40, 0.2g
MW: 360.96g/mol

Related products

See our catalog BioSciences for:

- IPTG (Isopropyl-Beta-D-Thiogalactopyranoside) #UP84853C, to induce the lacZ gene of β-Gal recombinent systems.
MW : 238.3 ; CAS : 367-93-1 ; Purity:>99%, Biotech grade ; Technical Sheet
-other fluorigenic β-Galactosidase substrates (Sampler kit #BM8400)
.Nitrophenyl-β-D-Galactopyranoside (oNPG, 2-NPG #556683 and pNPG, 3-NHP) #02493B, a chromogenic substrate that is converted by β-galactosidase to a soluble yellow product, that can be read at 410nm. This makes oNPG useful as a substrate for ELISA based assays.
MW : 301.26 ; CAS : 7493-95-0. Technical Sheet.
.β-Gal staining kit, Colorimetric blue staining #J2966
-substrates for other osidases (glucuronidase...)
-other reagents for cloning and nucleic acid amplification (D20-D66)

Ordering information

Catalog size quantities and prices may be found at http://www.interchim.com.

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Order on-line or Contact your local distributor

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