

Frequently Asked Questions

X-GAL

1. How X-GAL could be properly storage?

X-GAL is an hygroscopic and light sensitive powder. You must place X-GAL powder in a sealed desiccated container at 20°C that is not exposed to light.

2. Which is X-GAL stability?

X-GAL powder is stable for at least two years at -20°C, if not more while X-GAL solutions are stable for 12 months at -20°C protected from light. We recommend to distribute X-GAL solution in aliquots to avoid degradation by freeze/ thawing cycles.

3. How is prepared a X-GAL solution?

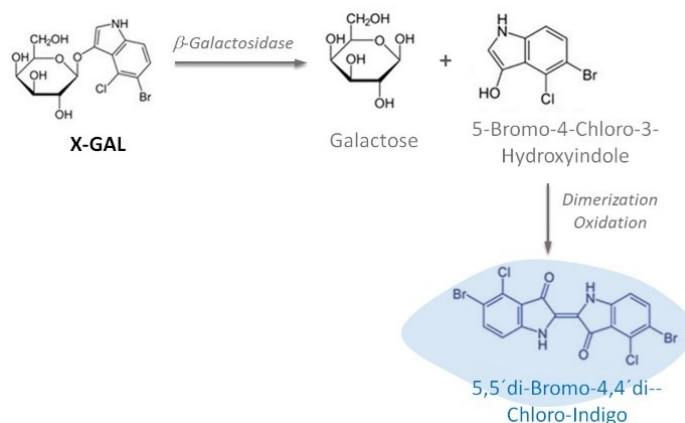
We recommend to prepare a solution at 20 mg/mL in N, N-dimethylformamide (DMF) (CAS 68-12-2) or dimethyl sulfoxide (DMSO) (CAS 67-68-5). Solutions in DMF are more stable than DMSO solutions. Store stock solution in dark glass or HDPE container protected from light at -20 °C.

4. Is it necessary to sterilize X-GAL solutions?

Sterilization is not required.

5. Has X-GAL Solution blue color?

No. X-GAL solution is a colorless liquid. β -galactosidase activity cleaves X-GAL releasing a compound which after dimerization and oxidation produces a blue insoluble product.



6. Does X-GAL influence on colonies blue intensity?

No. Blue intensity could be related with an improper distribution of X-GAL/ IPTG on the plates or with differences in β -galactosidase production due to differential colony growth.

7. When X-GAL solution should be discarded?

If X-GAL solution turns pink, it should be discarded. The appearance of pale blue or light yellow color of X-GAL Solution, ready-to-use, does not have influence on its quality.

8. How it is prepared X-GAL Blue/White selective plates?

X-GAL plates includes X-GAL, IPTG and an antibiotic resuspended in a culture media, generally Luria broth agar. X-GAL plates are usually prepared in two different ways. One way implies the addition of each component in melted LB agar media (see the table below) and pouring the mixture on sterile petri dishes. Protect from light X-GAL plates.

Components	Volume	Final Concentration
X-GAL Solution 2% (TBR0111)	250 μ L	0,5 mg/mL
IPTG 100 mM	134 μ L	134 μ M
Antibiotic	*	*
LB Agar Media	100 mL	1x

* Depends of each antibiotic.

In the second way, it is necessary to have LB plates with antibiotic. On the surface of the plate, add 40 μ L X-GAL 20 mg/mL and 40 μ L IPTG 100 mM. Spread on the plate with a sterile Drigalsky spatula. Allow the liquid absorption (~ 20 minutes) prior bacterial plating.

9. Can be used the white/ blue selection of recombinant clones y any bacterial cells?

No. Some bacterial strains have β -galactosidase activity naturally. Then it is necessary to use strains without β -galactosidase activity or with *lacZ* gene mutated. *lacZ*M15 mutation deactivates *LacZ* activity producing an inactive form of β -galactosidase¹. However, the β -galactosidase enzyme activity can be restored through α -complementation mechanism by introducing a plasmid carrying a *LacZ* alpha subunit into the *E. coli* strain, which therefore complements the truncated *LacZ* gene and produces an active β -galactosidase enzyme.

10. What are suitable strains to be used in white/blue screening selection?

The most used strains to select recombinant clones by white/ blue screening are

E.coli strain	Genotype
DH5 alpha	F, <i>endA1</i> , <i>recA1</i> , <i>glnV44</i> , <i>thi-1</i> , <i>relA1</i> , <i>gyrA96</i> (nal ^R), <i>deoR</i> , <i>nupG</i> , $\Phi 80lacZ\Delta M15$, $\Delta(lacZYA-argF)$, U169, <i>hsdR17</i> (rK ⁻ mK ⁺), λ^-
DH10B	F, <i>endA1</i> , <i>recA1</i> , <i>galE15</i> , <i>galK16</i> , <i>nupG</i> , <i>rpsL</i> , $\Delta lacX74$, $\Phi 80lacZ\Delta M15$, <i>araD139</i> , $\Delta(ara, leu)7697$, <i>mcrA</i> , $\Delta(mrr, hsdRMS, mcrBC)$, λ^-
JM109	<i>endA1</i> , <i>glnV44</i> , <i>thi-1</i> , <i>relA1</i> , <i>gyrA96</i> , <i>recA1</i> , <i>mcrB+</i> , $\Delta(lac proAB)$ e14- [<i>F'</i> <i>traD36 proAB+</i> , <i>lacI^q</i> , <i>lacZ\Delta M15</i>], <i>hsdR17</i> (rK ⁻ mK ⁺)
TOP10	F, <i>endA1</i> , <i>recA1</i> , <i>galE15</i> , <i>galK16</i> , $\Delta lacX74$, <i>nupG</i> , $\Phi 80lacZ\Delta M1$, <i>araD139</i> , $\Delta(ara, leu)7697$, <i>rpsL</i> (Str ^R), <i>mcrA</i> , $\Delta(mrr, hsdRMS, mcrBC)$, λ^-
XL1 Blue	<i>endA1</i> , <i>recA1</i> , <i>gyrA96</i> (nal ^R), <i>thi-1</i> , <i>relA1</i> , <i>glnV44</i> , <i>F'</i> [::Tn10 <i>proAB+</i> , <i>lacI^q</i> , <i>lacZ\Delta M15</i>], <i>hsdR17</i> (rK ⁻ mK ⁺)
XL10-Gold	<i>endA1</i> , <i>glnV44</i> , <i>recA1</i> , <i>thi-1</i> , <i>gyrA96</i> (nal ^R), <i>relA1</i> , <i>lac</i> , The, $\Delta(mcrA)183$, $\Delta(mcrCB, hsdSMR, mrr) 173$, tet ^R , <i>F'</i> [<i>proAB</i> , <i>lacI^q</i> , <i>lacZ\Delta M15</i> , Tn10 (Tet ^R Amy Cm ^R)]

¹ Yanisch-Perron C, Vieira J, Messing J, Chambers S, Prior S, Barstow D, Minton N, Gilbert W, Messing J, Messing J. Improved M13 phage cloning vectors and host strains: nucleotide. *Gene*. 1985;33:103–119. doi: 10.1016/0378-1119(85)90120-9.