

X-Gal – Frequently Asked Questions (5-bromo-4-chloro-3-indolyl-b-D-galactopyranoside)

The [X-Gal substrate](#) is metabolized by the enzyme β -Galactosidase into an insoluble blue precipitate. This technical note addresses some of the more common questions Gold Biotechnology has received over the last 26 years.

Handling and Storage of X-Gal

First, record the purchase date on your X- Gal bottle. X-Gal is stable at room temperature for short periods of time. In experiments conducted at GBT, we have left X-Gal at ambient temperature for over three weeks with no detectable loss of activity. For long term storage we recommend that you place the X-Gal in a sealed desiccated container at -20°C that is not exposed to light. **Always** warm the desiccator to room temperature for at least one hour prior to removing the X-Gal. X-Gal is hygroscopic and it is critical to avoid any condensation of water on the X-Gal crystals.

X-Gal Stability

If stored properly, dry powder X-Gal should be stable for at least two years, if not more. However, it is important to remember that with time, multiple users may access the X-Gal and the chances of improper handling increase. Since the price of X-Gal is insignificant compared to the overall cost of the experiment, we recommend that you consider turning over older X-Gal after a set period of time. X-Gal Solution is stable for 6-12 months at -20°C . However, frequent use will cause degradation of the solution over time and we recommend making new stocks every 2-3 months depending on use.

X-Gal Preparation

X-Gal is soluble in a number of solvents but the preferred solvent for biological experiments remains dimethylformamide (DMF). Some investigators prefer to use the solvent DMSO, but in our experience it is critical that the DMSO be relatively new and of the highest quality. We have consistently found that many problems associated with X-Gal experiments can be traced to the use of old or lower quality DMSO. Therefore, for most experiments we recommend that you dissolve X- Gal to a final concentration of 20 mg/ml in DMF.