

Beta-galactosidase activity staining in CNS
Per F. Chanut, 2001

1. Pre-chill your dissection medium (PBT, PBS, or Ringer's solution) and fix (1% gluteraldehyde in PBS) at 4°C.
2. Dissect the central nervous system (CNS) from 3rd instar larvae or adults in cold PBS. (Refer to dissection protocol for more details.)
3. Transfer each CNS into cold fix. Leave on ice for 20 minutes.
4. Wash with cold 1X PBS twice 10 minutes.
5. Transfer to pre-warmed (37°C) X-gal solution (6.7 μ l X-gal solution per 200 μ l staining solution) and incubate at 37°C. Depending on the strength of expression, the incubation period can vary from 15 minutes to overnight.
6. Wash with one change of PBS.
7. Mount in Glycerol (80% in 1XPBS).

Staining solution – 100 ml

100 ml	PBS
860 mg	Sodium chloride (NaCl)
20.3 mg	Magnesium chloride (MgCl ₂)
109 mg	Potassium ferricyanide (K ₃ [Fe(III)CN ₆])
140 mg	Potassium ferrocyanide trihydrate (K ₄ [Fe(II)CN ₆])

Combine ingredients and heat to 65°C until all ingredients solubilized.
Store at 4°C, protected from light. Warm to 37°C prior to use.

X-gal solution – 1 ml

1 ml	N, N Dimethyl formamide (DMF)
200 mg	X-gal

Mix until completely dissolved. Store at 4°C, protected from light. Warm to 37°C prior to use.