β-GAL/Magenta-Gal staining of LacZ Rhizobia in infected root hairs of *Medicago truncatula*

Reagents required

1. **Gluteraldehyde: 2.5%** (Thaw Gluteraldehyde solution at 4° C the night before and prepare solution for 50 mL and store at -20°. Storage/Stability Purified samples of 25% glutaraldehyde stored at -20 °C showed virtually no change in their UV absorbance characteristics even after 8 months. However, solutions are very heat sensitive.)

2. Z Buffer:

	REAGENTS	STOCK	REQUIRED		For 1L
1.	0.5 M Sodium Phosphate Buffer (pH 7.0)			100 mM	100 mL
	Sodium DiHydrogen Phosphate (Na_2HPO_4)	1M	61 mL		
	Sodium phosphate dehydrate (NaH_2PO_4)	1M	39 mL		
2.	Pottasium Chloride (74.55)	1M		10 mM	10 mL
3.	Magnesium chloride Heptahydrate (203.3)	1 M		1 mM	1 mL

3. Pottassium Ferro and Ferri Cyanide

	REAGENTS	STOCK	For 100 mL
1.	Potassium ferrocyanide $K_4[Fe(CN)_6]•3H_2O$	100 mM	3.92 g
2.	Potassium Ferricyanide $K_3[Fe(CN)_6]$	100 mM	4.22 g

4. X-Gal Solution

	REAGENTS	STOCK
1.	Z Buffer	880 µL
2.	Potassium Ferrocyanide	50 µL
3.	Potassium Ferricyanide	50 µL
4.	4% X-Gal in DMF (40 mg/mL)	20 µL

Procedure:

1. Transfer seedlings into 6 well plates containing 2.5% gluteraldehyde solution.
2. Keep plate in Vacuum for 5-8 minutes, till bubbles appear and tissue sinks. Slowly release vacuum and leave tissue in solution for 1 Hour at room temp.
3. Wash seedlings in Z Buffer thrice for 5 minutes each.
4. Add X-gal Solution to seedlings and keep plate in dark at 30 °C for 3-4 hours.
5. Remove staining solution and wash with Z buffer for 5 minutes repeatedly till background clears. If required, add 2-3 drops of NaEDTA 0.5 M per 5 mL solution to avoid fungal contamination.