

β -galactosidase Staining of Pre-Implantation Mouse Embryos

(Rivera lab)

(Adapted from R. Behringer's protocol)

STAIN - Make fresh with 975 μ l of **Stock 1** and 25 μ l of **Stock 2**.
If precipitate forms, discard and start again. Filter before use.

Stock 1: PBS Ca⁺⁺/Mg⁺⁺ free, pH 7.4 50.0 ml
Potassium Ferricyanide 82.0 mg
Potassium Ferrocyanide 106.0 mg
1M MgCl₂ 100.0 μ l
Filter using 0.45 μ m filter, store at room temperature.

Stock 2: 40 mg/ml X-Gal in DMSO or DMF. Store at -20 °C.

FIX Make fresh.
37% Formaldehyde 54.0 μ l
25% Glutaraldehyde 8.0 μ l
PBS Ca⁺⁺/ Mg⁺⁺ free, pH 7.4 + 10 mg/ml BSA 938.0 μ l
Filter using 0.45 μ m filter before use.

WASH PBS Ca⁺⁺/Mg⁺⁺ free, pH 7.4 plus 10 mg/ml BSA. Filter, store at 4 °C.
Refilter before use.

PROCEDURE

Set up a 60 mm dish with microdrops of 50 μ l covered with mineral oil.

Drop 1	Wash	5 min
Drop 2	Fix	10 min
Drop 3	Wash	10 min
Drop 4	Wash	10 min
Drop 5	Wash	10 min
Drop 6	Stain	1 hour to overnight at 37 °C.

Move the embryos singly (to avoid clumping) through drops with minimal transfer of solution between drops.