

Animal Cap Assay (AC assay) - Hiroki Kuroda

A Basic Technique for Embryo Manipulation

Note: This is a good first step in becoming a frog embryologist.

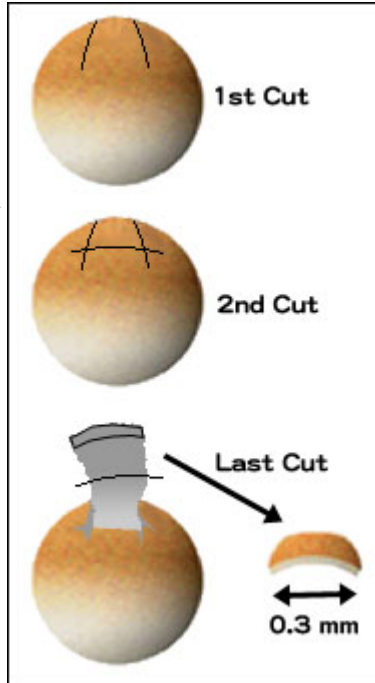
Note: 1x Steinberg's solution is used in every step.

- Remove the chorion membrane from the vegetal side. Note: it is better to make a small puncture on the vegetal side than in the animal side.
- Position embryo so that the animal side is facing up using the puncture on the vegetal side.
- Cut 3x3 mm² sized AC with sharp forceps.
- (Option) If you want to use treatment with TGFβ-related protein, 1x Steinberg's solution with 0.1% BSA is required.

(By *Gastromaster*)

Note. [*Gastromaster*](#) is a very useful tool for animal cap assay but is not good for transplantation and other manipulations.

- Cut 3x3 mm² sized AC with the yellow wire-holder at the lowest power of gastromaster; use an upward stroke.
Note. Removal of the cortical membrane is not necessary.



10x Steinberg's solution

NaCl 34g (580 mM), KCl 0.5g (6.7 mM), CaNO₃-4H₂O 0.8g (3.4 mM), MgSO₄-7H₂O 2g (8.3 mM), Kanamycin 0.1 g, Tris (MW=121) 6.0g (50 mM), adjust pH between 7.35-7.45 by HCl, adjust volume to 1 liter with H₂O and autoclave.

1x Steinberg's solution

Kanamycin 0.1g, 10x Steinberg's solution 100 ml, adjust volume to 1 liter with H₂O and autoclave.

1 x Steinberg's solution with 0.1% BSA

BSA 0.1 g in 100 ml of 1x Steinberg's solution, sterilize by filtration.