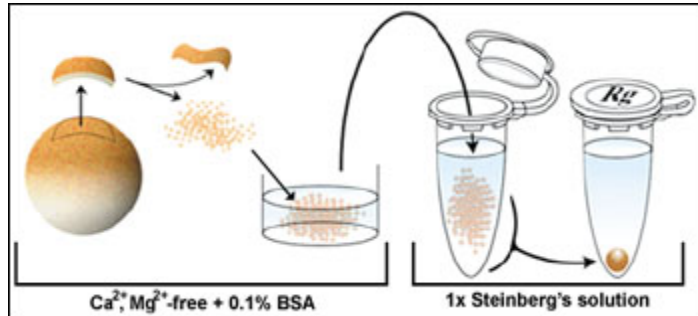


## Dissociation-and-reaggregation of animal cap cells – Hiroki Kuroda

Kuroda et al. *Genes Dev.* 19, 1022-1027 (2005)

**Note: This protocol is for making one reaggregate.**

**Note: Always use coated 3.5 mm sized plastic plate.**



### (Dissociation)

- Cut 10 animal caps at stage 9 in Ca<sup>2+</sup>, Mg<sup>2+</sup>-free 1 x Steinberg's solution with 0.1% BSA in plate A.
- Move 10 animal caps into plate B with same solution.
- Wait for 2-3 min.
- Dissociate by gentle pipetting.
- Gently rotate plate B 50-100 times by hand to gather most cells to center of plate.
- (Option1: Neural induction) Move cells into the same solution on plate C and culture for more than 3 hours at RT or until sibling embryos reach stage 13. Sox2 and NCAM are good marker for neural tissue, and cytokeratin for epidermis.
- (Option2: Protein treatment) Move cells into protein solution in Ca<sup>2+</sup>, Mg<sup>2+</sup>-free condition on plate C and culture until scheduled time.

### (Reaggregation)

- Move dissociated cells into 1.5 ml siliconized tube.
  - Centrifuge (1000 rpm, at RT, 10 sec).
  - Remove as much of the supernatant as possible.
  - Add 1 ml of new 1 x Steinberg's solution (no BSA).
  - Centrifuge (1000 rpm, at RT, 10 sec).
  - (Option) If you are using cells for protein treatment at higher concentration, you should do this wash step 2-3 times.
  - Culture precipitate in tube.
- Note: Reaggregate is formed 2-3 h after culture. If you want to culture longer (e.g., 2-3 days), you should change solution every 8-12 h.

### 10x Steinberg's solution

NaCl 34g (580 mM), KCl 0.5g (6.7 mM), CaNO<sub>3</sub>-4H<sub>2</sub>O 0.8g (3.4 mM), MgSO<sub>4</sub>-7H<sub>2</sub>O 2g (8.3 mM), Kanamycin 0.1 g, Tris (MW=121) 6.0g (50 mM), adjust pH between 7.35-7.45 with HCl, adjust volume to 1L with H<sub>2</sub>O and autoclave.

### 1x Steinberg's solution

BSA 1g, Kanamycin 0.1 g, 10 x Steinberg's solution 100 ml, adjust volume to 1L with H<sub>2</sub>O and autoclave.

### 10 x Ca<sup>2+</sup>, Mg<sup>2+</sup>-free Steinberg's solution

NaCl 34g (580 mM), KCl 0.5g (6.7 mM), Kanamycin 0.1 g, Tris (MW=121) 6.0g (50 mM), adjust pH between 7.35-7.45 with HCl, adjust volume to 1L with H<sub>2</sub>O and autoclave.

### 1x Ca<sup>2+</sup>, Mg<sup>2+</sup>-free Steinberg's solution with BSA

Kanamycine 0.1g, 10x Ca<sup>2+</sup>, Mg<sup>2+</sup>-free Steinberg's solution 100 ml, adjust volume to 1L with H<sub>2</sub>O and sterilize by filtration.

### Coated 3.5 mm plates

Take Poly (2-hydroxyethyl methacrylate) 12% in (poly Hema) ethanol and dilute in an equal volume of 100% EtOH. Mix well and coat the plate well with a q-tip. Allow the plate to dry before using.