

SGN dissociation

Acronyms or shorthands:

EBSS Earle's Balanced Salt Solution

HBSS Hank's Balanced Salt Solution

Recipes:

Collagenase (500U/mL): chilled 10 mL HBSS in collagenase (brown bottle), vortex and rotate in 4°C for **10 min**, up to 50 mL with more chilled HBSS (in 50-mL falcon tube). Aliquot and store in -20°C.

Ovomucoid inhibitor: 32 mL EBSS + albumin ovomucoid inhibitor mixture (until dissolved). Store at 4°C for up to 10 wk. Warm up before use.

Preparing for dissociation:

- Incubate pre-made collagenase aliquot and ovomucoid solution in 37°C water bath.
- 20 U/mL Papain (add 5 mL to bottle) – discard any left over
- DNase (add 500 µL of EBSS to one vial)
- Tube A: 2.7 mL EBSS + 300 µL Ovomucoid + 150 µL DNase (*optional*)
- Tube B: 4 mL Ovomucoid
- Keep everything in the 37°C water bath.

Dissociation:

1. Dissect cochleas out and chop into pieces in EBSS.
2. Add cochlea to tube containing 1 ml Collagenase. Rock at 37°C for **20 min**.
3. Pellet for **5 min** at 3 setting. Remove collagenase and add 2 mL Papain, then 100 µL DNase (*optional*). Rock at 37°C for **20 min**.
4. Only small chunks of cochlea should remain in suspension at this point. Pellet for **5 min** at 3 setting. Remove Papain, add 2 mL of EBSS + ovomucoid + DNase solution. Resuspend gently with 4-10 pipetting.
5. Run it through a 20-micron sorter (use a petridish).
6. Layer on top of 4 mL ovomucoid.
7. Spin for **3 min** at 4 setting.
8. Remove all but the last 30 or so µL of solvent. Add 30 µL warm EBSS, triturate gently, plate on coverslip / slide / well.