

***Aiptasia* Host Cell Maceration Protocol**

Protocol authors: Elisha Wood-Charlson, Virginia Weis

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1. Incubate animal in Ca⁺⁺free SW ~30min
2. Cut tentacles from animal, transfer to well slide, sealed w/ poly-lys coverslip
3. Cut further into barrels and smooch. The smaller the better.
4. Add 0.05% collagenase in 0.1M Na phosphate buffer containing 3% NaCl and 0.004% CaCl, pH 7.4 (this can be frozen in aliquots for future use)
5. 30 min shaking gently
6. 30 min w/o shaking to settle cells
7. Melt 1% LMT agarose in Ca²⁺free SW, keep at ~33°C
8. Pour off 0.05% collagenase solution
9. Fill well with Hoescht stain solution (see below)
10. 30 min w/o shaking, in dark
11. Pour off dye, dry around edges of well
12. Add THIN layer of 1% LMT agarose, spread while adding - suck off extra
13. 10 sec at 4° C.
14. Add Ca²⁺SW to fill well

Ca⁺⁺free SW

NaCl	11.74g
MgCl ₂	2.49g
Na ₂ SO ₄	1.96g
KCl	0.332g
NaHCO ₃	0.096g
H ₃ BO ₃	0.013g
up to 500mL dH ₂ O	
final ~30 parts/thousand	
pH ~ 8.0	

Hoescht stain solution

Hoescht stock: 0.5 mg/ml

Add 1 ul of this stock per 1 ml of Ca⁺⁺free SW