# Gentle Cell extraction from rock samples.

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1 Works for me Share This protocol is published without a DOI.

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ABSTRACT

Method for extracting cells in a gentle manner from rocks for downstream single cell sorting and sequencing. Starting material for the protocol is rock in a 15 mL centrifuge tube, stored frozen in 5 mL of Glycerol TE. GlyTE stock recipe:
1. Mix the following:
a. 20 mL 100x TE pH 8.0
b. 60 mL deionized water
c. 100 mL molecular-grade glycerol (use a syringe)
2. Pass the glyTE stock through a 0.2 micrometer pore size filter
3. Store at -20ºC.

PROTOCOL CITATION

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Sample is started from 5 g of rock, preserved in 5 mL of glycerol TE in a 15 mL centrifuge tube.

1 Add EDTA to 15 mL tube ( containing 5 mL glyTE and 5 g crushed rock), add EDTA for final concentration of 10 mM.

2 Vortex two tubes at time horizontally in tube holder of the Vortex-Genie-2. Vortex for 5 min at speed 7 (too high of a speed could allow the rock particles to cause damage to the cells)

3 Sonicate, two tubes at a time in ice water, for 1 Minute

4 Centrifuge at 1,000 RPM for 5 minutes in the bench-top centrifuge in the Emerson Lab to pellet rock particles. Transfer the supernatant to a fresh 15 ml Falcon tube

5 Centrifuge the supernatant 2ml at time @ 10,000 RPM for 5 minutes in 2 x 2 ml centrifuge tubes. One tube for sorting, and another as replicate or microscopy. Approximately 2.5 ml will be pelleted in each tube over two rounds of centrifugation (2 ml, 2ml, ~ 250 ul). Remove supernatant each time, with caution, using a pipette. Do Not Pour Off Supernatant, cellular material is not always well pelleted and can be lost easily.

6 a. For one tube (for later sorting) Resuspend the pellet in 1ml of 1X GlyTE. Can dilute with 1X PBS. Store in freezer.

b. For second tube (for microscopy) – stain with SYBR Green, and filter on 0.2 um polycarbonate filter, rinse and dry filter, then mount on glass slide with Citifluor antifade mounting solution. Count cells, or store slide at 4C until ready to count cells