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Chyme sampling from the digestive tract of *Octopus maya*

 In 1 collection

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We use this protocol and it's working

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Abstract

This protocol details the chyme sampling from the digestive tract of *Octopus maya*.

Materials

Materials and equipment

- Surgical tools: scissors and clamps
- Centrifuge capable of reaching  13370 x g
- Tissue homogenizer (e.g., ULTRA-TURRAX IKA T18 basic)
- Liquid nitrogen
- Cryogenic storage containers ( -40 °C freezer)
- Eppendorf tubes (1.5 mL or 2 mL)
- Protective equipment: gloves and lab coat



Preparation

- 1 Ensure all tools and equipment are clean and sterilized before starting.
- 2 Prepare the workspace with necessary materials, ensuring easy access to surgical instruments, centrifuge, liquid nitrogen, and Eppendorf tubes.
- 3 Use proper personal protective equipment (PPE) throughout the procedure.

Dissection of the Digestive Tract

- 4 Carefully dissect the digestive tract of *Octopus maya*, ensuring the integrity of each section.
- 5 Identify and separate the following sections:
 - Anterior salivary glands (BM)
 - Crop (Cr)
 - Stomach (St)
 - Caecum (Ca)
 - Digestive Gland (DG)
- 6 Use surgical clamps to isolate and prevent mixing of digestive contents between sections.

Sampling of chyme

5m

- 7 Open each section carefully to access the chyme.
- 8 Transfer the chyme into labeled Eppendorf tubes.
- 9 Centrifuge the chyme samples at  13370 x g, 00:05:00 to separate solids from the supernatant.
- 10 Collect the supernatant and immediately freeze it in liquid nitrogen.

5m





11 Store the frozen samples at  -40 °C until analysis.



Extraction of enzymes from the buccal mass (BM)

5m

12 Dissect the anterior salivary glands.

13 Liquefy and homogenize the glands using a tissue homogenizer (e.g., ULTRA-TURRAX IKA T18 basic) at an appropriate speed for uniform homogenization.

14 Transfer the homogenized sample into an Eppendorf tube.

15 Centrifuge the sample at  13370 x g, 00:05:00 to obtain a clear supernatant.

5m



16 Freeze the supernatant in liquid nitrogen immediately after centrifugation.

17 Store the sample at  -40 °C until further analysis.



Note

Notes and precautions:

- Ensure the surgical clamps are properly placed to prevent cross-contamination of digestive contents.
- Work quickly when handling biological samples to preserve enzyme activity.
- Properly label all tubes with the section of the digestive tract and the sample type for accurate identification.
- Use liquid nitrogen with caution and follow appropriate safety protocols to avoid burns or injuries.

Storage:

Samples should remain frozen at  -40 °C to preserve enzyme and metabolite integrity until the time of analysis.

