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Isolation of Parasitoid Wasp Venom

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We use this protocol and it's working

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Abstract

This protocol has been developed for the efficient isolation and purification of whole venom from parasitoid wasps. Venom purified through this protocol is suitable for use in biochemical and cell biological assays, and for characterization by mass spectrometry. Venom is typically isolated from 50-100 female wasps, depending on the needs for the downstream application.

Materials

Solutions and reagents:
- PBS [137 mM NaCl, 2.7 mM KCl, 10 mM Na2HPO4, 1.8 mM KH2PO4, pH 7.4]
- HALT™ protease inhibitor (ThermoFisher Scientific)
- Prepare the PBS-HALT solution by diluting HALT™ protease inhibitor into PBS. Gently mix the sample with a pipette to ensure homogeneity of protease inhibitor.
- PBS-HALT should be prepared fresh daily for venom isolation. Prepare 200 µL for each sample to be dissected.

Consumables needed:
- Gel loading tips (LC1001, Invitrogen™)
- Kimtech Science™ Kimwipes™
- 2 sharp forceps (e.g. Dumont #55 Forceps)
- 0.22 µm PES syringe filter (SFPES004022N, Membrane Solutions)
- Pestle Homogenizer + Pestles (ThermoFisher Scientific)
- Eppendorf tubes (Sigma-Aldrich)
- Spot plate with depression wells for use as a dissection plate (4132, US Geo Supply)

Laboratory equipment:
- Benchtop centrifuge capable of 8000 x g
- Stereo dissecting microscope
- CO_2 apparatus for wasp anesthesia

Preparation for dissection

1 To prepare for venom extraction, you will first prepare a containment well to hold samples following dissection. Pipet 150 µL of PBS-HALT into the open cap of a 1.5 mL Eppendorf tube, and place the tube in ice such that the open cap is in contact with the ice.

2 Prepare your dissection plate. Add ¾ volume of PBS to each well of a clean spot plate with depression wells.

3 Prior to dissection, wasps should be anesthetized with CO_2 within their containment tubes, the timing of which will be variable and determined by the efficiency of your CO_2 delivery system. Wasps may be maintained on a CO_2 pad for no more than 15 minutes. To prepare wasps for dissections, move them in batches of 10 to a dissection well.

Venom apparatus dissection

4 To begin each dissection, hold the body of the wasp with one set of forceps and gently grip the ovipositor with the second set of forceps. To extract the venom apparatus, gently tug on the ovipositor to liberate the apparatus tissue from the body of the wasp.

5 Use a clean Kimwipe to blot excess PBS from the forceps prior to placing the dissected apparatus into the Eppendorf containment well. Discard the body of the dissected wasp, and repeat until the desired quantity of apparati are dissected.

Purification of whole venom

6 To prepare purified whole venom for downstream experimentation, the Eppendorf cap is gently closed, and apparati are spun in a desktop centrifuge at 8,000 x g for 5 minutes at room temperature. This step will result in a tight pellet of apparati at the bottom of the tube.

7 Next, to homogenize tissue, a pestle homogenizer is used to grind the apparati and release venom into the supernatant of the sample. This homogenization is performed in short pulses of 2 seconds under non-lysing conditions in PBS-HALT.

8 To pellet cells, cellular debris, and any remaining tissues, the homogenized sample is incubated on ice for 5 minutes and centrifuged at 1,000 x g for 10 minutes at room temperature.

9 Following centrifugation, the supernatant is removed from the pellet, transferred to a fresh 1.5 mL Eppendorf tube and may be stored at 4 °C.

10 Due to aggregation of venom vesicles that sit for prolonged periods of time at 4°C, filtration is required to minimize this aggregation phenotype. Venom can be filtered using a 0.22 µm PES syringe filter and used for downstream applications.