



VERSION 1

MAR 27, 2024

OPEN ACCESS



DOI:

[dx.doi.org/10.17504/protocols.io.yxmvme7n6g3p/v1](https://dx.doi.org/10.17504/protocols.io.yxmvme7n6g3p/v1)

**Protocol Citation:** Tatum S Katz, Alyssa Byer, Renwei Chen 2024. *Drosophila melanogaster* inoculation with Bd. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.yxmvme7n6g3p/v1>

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**Protocol status:** In development  
We are still developing and optimizing this protocol

## *Drosophila melanogaster* inoculation with Bd V.1

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### ABSTRACT

Methods to inoculate *D. melanogaster* with *Batrachochytrium dendrobatidis*.

For manuscript *Drosophila melanogaster* is a possible vector of *Batrachochytrium dendrobatidis* under review at PLOS One.

**Created:** Mar 27, 2024

**Last Modified:** Mar 27, 2024

**PROTOCOL integer ID:** 97460

**Funders Acknowledgement:**

NIH Ecology and Evolution of  
Infectious Diseases

Grant ID: R01GM135935

University of California Santa  
Barbara California Alliance for  
Minority Participation

## Protocol

- 1 Plate 0.5ml of treatment ( $10^6$  zsps per fly or DI water) onto each agar plate
- 2 Allow plates to dry so that no water is puddled on the surface
- 3 Place 10 flies onto each plate
- 4 Seal plates with parafilm and incubate at room temperature for 24 hours
- 5 Transfer flies into fly vials (keeping all flies from the same plate together in the same vial)

- 6 Euthanize flies in the freezer at desired time point (within 5 days). Or, use the living inoculated flies for another protocol
  
- 7 If you want to test the difference between washed and unwashed flies, you may wash a group of up to 5 flies at a time in PBS buffer using 500ul per fly prior to DNA extraction.
  
- 8 Inoculated flies are now ready for DNA extraction or other methods.