

Western Blot Sample Prep- Whole Drosophila Embryos

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Abstract

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Before start

It is important to plan the timing of the embryo collection in advance. To select a specific genotype, be sure to cross in the selectable balancer that will allow you to distinguish mutants from non-mutants. To prevent the collection of unfertilized animals into sample, it is helpful to select animals that have developed far enough through embryogenesis that auto-fluorescence of the gut can be observed.

Example: To collect stage 17 Mcr[P1] animals: Setup a four hour collection and age for 17 hours at 25 degrees Celsius. For homozygous mutants, collect non-YFP animals and select only animals that have auto-fluorescence in the gut.

Protocol

Step 1.

Collect 100 embryos and place into eppendorf tube (1.5ml) containing 50ul of embryo wash

 **AMOUNT**

50 µl Additional info:

Step 2.

Add 3 drops of bleach and let sit for 3 mins.

 **AMOUNT**

150 µl Additional info:

 **DURATION**

00:03:00

Step 3.

Raise volume to 1.5ml using dH2O

Spin down for 1min

 **AMOUNT**

1500 µl Additional info:

 **DURATION**

00:01:00

Step 4.

Remove solution
Add 1.5ml of dH₂O
Spin down 1min

 DURATION

00:01:00

Step 5.

Remove solution
Add 1.5ml of dH₂O
Spin down 1 min

 AMOUNT

1500 µl Additional info:

 DURATION

00:01:00

Step 6.

Remove solution with micropipette
Add 50ul of 1X PBS

 AMOUNT

50 µl Additional info:

Step 7.

Add 50ul of 2X SDS SB

 AMOUNT

50 µl Additional info:

Step 8.

Homogenize with pestle and 27ga needle

Step 9.

Boil for 5 mins at 98 degrees C

 DURATION

00:05:00

Step 10.

Store at -20 degrees C