Dissecting and Immunostaining Drosophila melanogaster Egg Chambers

Rob Ward's Lab

Abstract

Drosophila melanogaster females' productive system has been an excellent model in studying many developmental events such as stem cell specification and cell migration. The ovaries are composed of a chain of egg chambers starting with the germaruim that buds of stage 1 egg chamber after encapsulated it with follicle cells. Then, stage 1 egg chamber goes under 14 developmental stages. This protocol provides the basic method for both dissecting and immunostaining ovaries. Each step is critical. This protocol is modified from http://www.ncbi.nlm.nih.gov/pubmed/8162854

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Protocol

Prepare the reagents

Step 1.

1- The fix:
4% Paraformaldehyde
1X PBS
Place 10 ml of 1X PBS in the microwave just to boil.
Add the .4 paraformaldehyde
Add 4 drops of 2M NaCl (make sure that Paraphormaldehyde is completely dissolved)
Add one drop 12ml HCL to adjust the PH (7.5-8)
2- The block :
10 ml of 1X PBS
10µl/ml Normal Donkey Serum
10µl/ml 10X Triton

AMOUNT
10 ml Additional info:
DURATION
00:05:00

Dissection

Step 2.

1- Set up the dissection microscopy.
2- Put the depression slide on it.
3- Put three drops of 1X PBS on the slide to maintain the PH and keep the egg chambers hydrated.
4- Anesthetize the flies by using CO2 bed.
5- Hold the fly from its wings with a size 5 tweezer.
6- Place it on the slide.

7- With your left hand hold the fly from its thorax and with your right hand gently grap the tip of the abdomen and pull it out (be sure to not damage the ovaries)
8- Squeeze the abdomen gently until the pair of the ovaries comes out.

AMOUNT
60 µl Additional info:

REAGENTS
1X PBS (Phosphate-buffered saline) by Contributed by users

DURATION
00:10:00

prepare for immunostaining

Step 3.
Transfer the ovaries from the depression slide into the fix using transfer piptte.

DURATION
00:20:00

prepare for immunostaining

Step 4.
Wash the ovaries 3X with 1X PBS using transfer piptte.

prepare for immunostaining

Step 5.
Transfer the ovaries into the block using the transfer piptte.

DURATION
00:30:00

immunostaining

Step 6.
After blocking the ovaries, transfer them into a microtube that is filled with fresh block add the primary antibody over night at 4C.

DURATION
00:02:00

immunostaining

Step 7.
Next morning:
1- Remove the block
2- Wash the ovaries 3X 1X PBS
3- Add block
Rock at RT for 20 mins

DURATION
00:30:00

immunostaining

Step 8.
After 30 mins
1- Remove the block
2- Wash one time with 1X PBS
3- Add block
4- Add secondry antibodiy
Be sure to cover the tube with foil to prevent the interaction between 2nd antibody with the light.
Rock at RT for 2-4 hours

DURATION

04:00:00

immunostaining

Step 9.

After secondray staining:
1- Remove the block
2- Wash 3X with 1X PBS
3- Add block
Rock for 30 mins at RT

DURATION

00:30:00

immunostaining

Step 10.

After washing the non- specific secondary antibody:
1- Wash one time with 1X PBS

mounting and visualizing

Step 11.

1- By using glass piptte, transfer the ovaries into a regular slide.
2- Try to remove most of the PBS but leave a little to keep the egg chamber moisturized.
3- Put three drops of mounting media and place the cover slip on top of the ovaries.
4- Heal the edges of the cover slip with a nail polish
5- The ovaries are ready to be visualized.

AMOUNT

30 µl Additional info:

DURATION

00:05:00

Warnings

PBS and Block should be fresh.