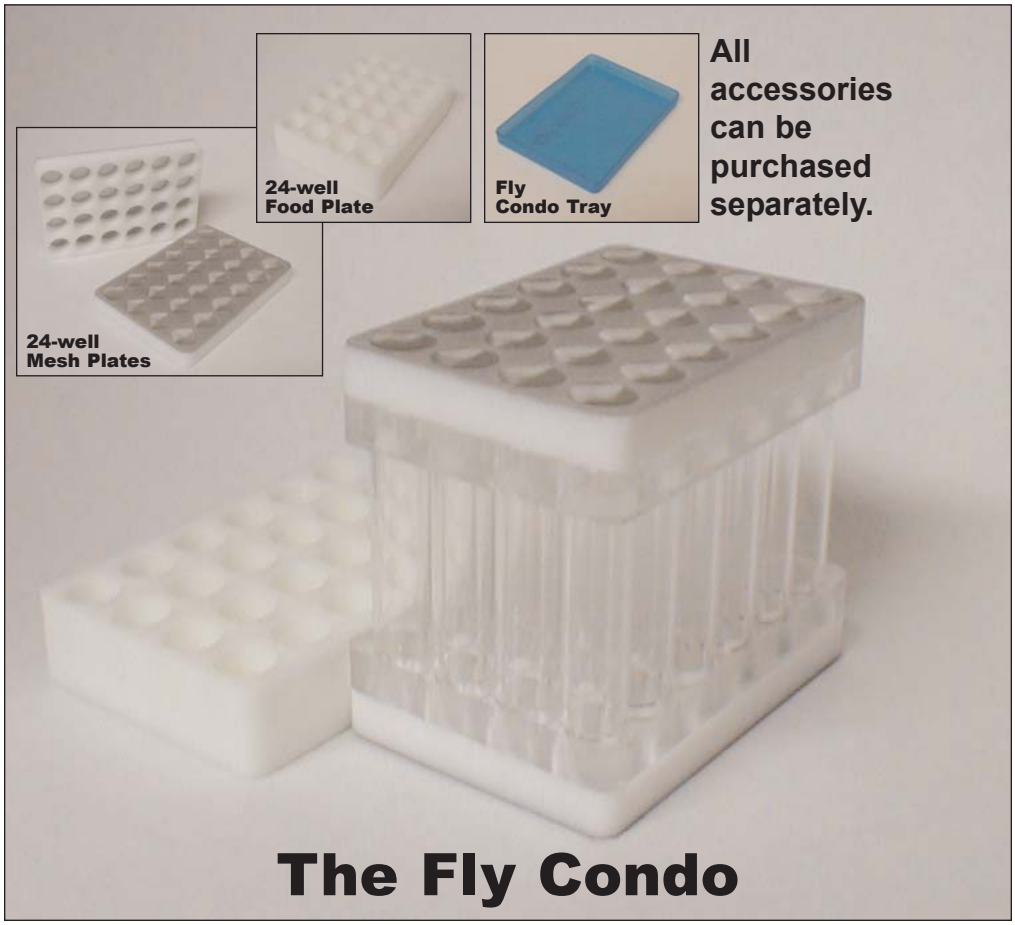
The Fly Condo™

For High Throughput Embryo Collection and/or Tissue Processing for Immunohistochemistry, In Situ Hybridizations and DNA Staining

The Fly Condo features 24 independent chambers, each connected to one another in a single, easy-to-handle cluster. This unique design facilitates the researcher to conduct 24 different experiments simultaneously under identical conditions. The Fly Condo can be used for high throughput collection of Drosophila embryos and/or processing of a variety of tissues for immunohistochemistry, in situ hybridizations and DNA staining. Thus the Condo is an excellent tool for genetic screens where phenotypes can only be visualized by embryo or tissue processing. The Fly Condo can also be used for whole-organism drug screening where chemical compounds are fed directly to larvae or adults. The Fly Condo footprint (8 x 11.6cm) fits on top of the **FlyStuff**™ CO_2 Flowbed or most other rectangular CO_2 staging apparatus, thus facilitating the addition of flies to each of the chambers and/or subsequent collection of adult flies from the chambers. Both the 24-well Stainless Steel Mesh Plates and the 24-well Food Plates are made of durable HDPE for resistance to most organic solvents, bleach and acids. Seal Plate polyester-based adhesive sealing films can be used for plate storage. Visit **www.flystuff.com** to view ***The Fly Condo Methods and Protocols***. Our protocols can easily be modified in order to fit your particular needs.



Cat #	Description	List Price	Contract Price
59-110	Fly Condo, Complete*	400.00	299.90
59-111	24-well Mesh Plates, 2 Plates/Unit	110.00	84.50
59-112	24-well Food Plate, 1 Plate/Unit	50.00	34.50
59-113	Condo Trays**, 10 Trays/Unit	10.00	6.50
22-167	Sealing Film, 100 Films/Unit	60.00	48.00

Complete Unit Includes: Chamber Cluster; 24-well Stainless Steel Mesh Plates (2); 24-well Food Plate (1); Condo Trays (10)

**** Fly Condo Trays are pipet tip tray lids which come with the following MBP Pipet Tip Trays: ART® 10, ART® 10 Reach, MBP 10, MBP 10 Reach (Doc Frugal Scientific is a Full-Line MBP Distributor)***

Visit www.flystuff.com for Fly Condo methods and protocols.

The Fly Condo was designed in collaboration with Giovanni Bosco, Ph.D., University of Arizona Cancer Center

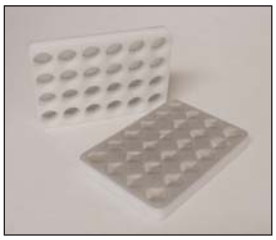
Doc Frugal
SCIENTIFIC
CORPORATION
800.789.5550
www.flystuff.com
flystuff@docfrugal.com

The Fly Condo™
Methods and Protocols

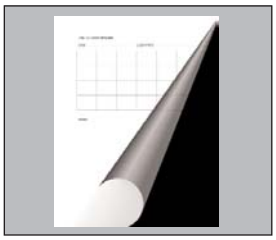
by Giovanni Bosco, Ph.D.

Fly Condo Accessories:

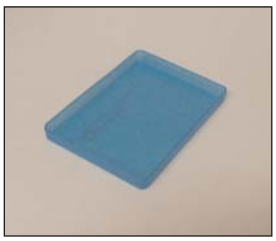
24-well Food Plate



24-well Mesh Plates



Fly Condo Template



Fly Condo Tray

I. Getting Adult Flies in and out of the Condo

1. Place one of the 24-well Mesh Plates onto the Condo chamber so that it fits snuggly.
2. Since the Condo is symmetrical, it is helpful to mark with a marker one of the corner chambers as #1 as it will help you keep track of what flies are in what chamber. Use the template below to note genotypes as you load flies into their chambers.
3. Invert the Condo onto an active fly CO_2 pad and using a small funnel place adult flies into each of the individual chambers. The flies will fall to the bottom were they are in direct contact with the CO_2 flow. This is ideal for experiments where many mutant fly lines will be used for embryo collections or drug screening, since the flies remain anesthetized by the CO_2.
4. For embryo collections:
a. When all the desired flies are placed into their individual chambers place the second 24-well Mesh Plate snugly onto the Condo chamber to seal it so that it prevents the flies from escaping. Tape can be used but normally it is not necessary to keep the bottom and top well-screens from falling off.
b. Remove from the CO_2 pad and allow flies to wake up.
c. Prepare a Condo Tray with grape juice agar and smear a thin film of wet yeast paste onto it. Firmly press the Condo Tray with wet yeast onto the 24-well Mesh Plate bottom so that the yeast oozes through the mesh into the chamber side of the of the mesh and is accessible to the flies for feeding.
d. Secure the Condo Tray to the Condo with tape.
e. Replace Condo Tray every day and monitor embryo production. To replace the Condo Tray: prepare a fresh grape agar plate as above; remove tape from old Condo Tray, place the fresh plate on the Condo and secure it. Embryos that are deposited into the chambers can be processed as described below.
5. For drug screening in adults:
a. The 24-well Food Plate is prepared with instant Drosophila food and the chemical compound of choice in each individual well. Normally, instant Drosophila food with or without yeast is mixed directly into each well with a chemical compound that one wishes to feed to the adult fly. Use a piece of tape or marker to denote chamber #1 as the Condo is symmetrical, and note the order of compounds on the template provided on reverse.
b. When all the desired flies are placed into their individual chambers place the 24-well Food Plate snugly onto the Condo chambers so that it prevents the flies from escaping.
c. Adult phenotypes elicited by drugs can be monitored by inverting the Condo onto a CO_2 pad and knocking out the flies so that they may be viewed/scored on a stereo microscope, moved to a fresh Condo, or processed for dissection or enzymatic activity.

II. Drug Screening in Larvae

1. Prepare the 24-well Food Plate with instant Drosophila food and the desired compound in each individual well, noting the orientation of the dish using the Fly Condo Template.
2. Collect embryos from a population cage from the desired fly stock.
3. Place approximately equal numbers of embryos in each well directly on top of the food/drug mixture. This can be done either by picking embryos up with a brush or cutting small slabs of agar from the plate upon which the eggs were deposited.
4. Place the Condo chambers onto the 24-well Food Plate with embryos and seal the other end with a 24-well Mesh Plate.
5. Place the Condo in the desired environment and allow the embryos to develop within the chambers. Since the volume of food is small it may dry out quickly. Therefore, an incubator or chamber with humidity control is recommended for long feeding experiments. Larvae and/or adult phenotypes can be scored at any time.

III. DAPI Staining of Embryos

Materials:
- dH_2O, 2 liters
- 50% Bleach (made fresh)
- 0.02% Triton X-100
- MeOH
- Heptane
- Fly Condo Trays ~25 **
- Shaker
- PBT (1xPBS w/ 0.005% Triton X-100)
- 50% MeOH/50% Heptane
- 90% MeOH/10%PBT
- 75% MeOH/25%PBT
- 50% MeOH/50%PBT
- 25% MeOH/75%PBT
- DAPI stock (100µg/ml in 100% EtOH)
- 70% Glycerol/30% PBS
- 50% Glycerol/50% PBS
- Paper towels for blotting

** Fly Condo Trays hold ~35ml of liquid when a 24-well Mesh Plate is inserted. Fly Condo Trays can be used as lids while washes are taking place or for long term storage.

1. Remove 24-well Mesh Plate with embryos from agar plate as described above. Using long forceps, pick up the 24-well Mesh Plate and place embryos immediately in a clean Condo Tray and wash with ~35 ml of dH_2O.
2. Wash 3x w/ dH2O or until all yeast is gone. Blot onto paper towels in between all washes.
3. Remove all dH_2O, add 50% bleach (made fresh). Swirl or shake ~5 min. Look for loss of appendages.
4. Remove bleach and place in 0.02% Triton X-100.
5. Wash 3x w/ 0.02% Triton X-100.
6. Wash 2x 3 min. w/ dH_2O.
7. Place into 50% MeOH/50% Heptane.
8. Shake hard (200 rpm) for 4 min.
9. Wash 3x 1min. in 100% MetOH.

10. Leave in 100% MetOH overnight at 4ºC, or incubate in MetOH for 3hrs at room temp. At this point embryos can be stored at 4ºC for weeks.
11. Wash 2x w/ 90% MetOH/10%PBT for 2 min.
12. Wash 1x w/ 75% MetOH/25%PBT for 2 min.
13. Wash 1x w/ 50% MetOH/50%PBT for 2 min.
14. Wash 1x w/ 25% MetOH/75%PBT for 2 min.
15. Wash 2x w/ PBT for 5 min.
16. Wash 1x w/ 1ml PBT and 1µg/ml final of DAPI for 10 min.
17. Wash 2x w/ PBT for 10 min.
18. Drain all PBT and add 50% Glycerol/50% PBS.
19. With a wide orofice (or cut-off) 200µl pipet tip place on slide, cover with large cover-slip and seal with clear nail polish.

IV. Immunofluorescence Staining of Embryos

1. Remove 24-well Mesh Plate with embryos from agar plate as described above. Using long forceps, pick up the 24-well Mesh Plate and place embryos immediately in a clean Condo Tray and wash with dH_2O.
2. Wash 3x w/ dH_2O or until all yeast is gone. Blot onto paper towels in between all washes.
3. Remove all dH_2O, add 50% bleach (made fresh). Swirl or shake ~5 min. Look for loss of appendages.
4. Remove bleach and place in 0.02% Triton X-100.
5. Wash 3x w/ 0.02% Triton X-100.
6. Wash 2x 3 min. w/ dH_2O.
7. Fixation:
(a) Fix for 15-20 minutes with gentle agitation in 1:1 solution of 4% EM-grade formaldehyde in PBS and heptane. Place in 1:1 solution of methanol:heptane and shake embryos for 4 minute at high speed to remove vitelline membrane. Place in methanol and fix in methanol over night at 4°C or 3 hours at RT.

or

(b) Fix directly with methanol: Place in 1:1 solution of methanol:heptane and shake embryos for 4 minutes at high speed to remove vitelline membrane. Place in methanol and fix in methanol O/N at 4°C or 3 hours at RT.
8. Rehydrate embryos - 5 minutes each in 90:10, 75:25, 50:50, 25:75, methanol: 1XPBS; one rinse in 1XPBS. Wash in antibody WASH solution for 30 minutes on shaker.
9. Block for 1 hour in BLOCK solution on shaker.
10. Incubate O/N with primary antibody in DILUENT at 4°C on shaker (cold room)
11. Wash embryos 2x quickly and then for 4x 15 minutes with WASH (i.e. 1 hour total - longer is ok)
12. Second block (this seems to be optional).
13. Incubate with secondary antibody in DILUENT for 3 hours at room temp. or O/N at 4°C (shaker) (general: FITC, Texas Red 1:200; Cy3 and Cy5 1:400).
14. Wash embryos 2x quickly and then for 4x 15 minutes with WASH (1-2 hrs).
15. Wash embryos in 1XPBS 10 min.
16. Stain with DAPI, 1:100 dilution of stock (100µg/ml) to 1µg/ml final for 10 min., and wash 10 min. in PBS. Mount in Vectashield or 50% glycerol- no need to dehydrate (Do not use Vectashield with Cy2).

WASH
1X PBS
0.2% BSA
0.1% Triton X-100

BLOCK
1% BSA
0.3% Triton X-100
10% donkey or goat serum
1mg/ml RNase A
1X PBS

DILUENT
0.1% BSA
0.3% Triton X-100
10% donkey or goat serum
1X PBS

FORMALDEHYDE FIX
4% Formaldehyde
1X PBS

V. Using the 24-well Mesh Plate alone for staining/labeling wholemount tissues

The 24-well Mesh Plate can be used for processing tissues with your existing protocol--now you can do 24 different mutant lines at the same time. Below is one example where BrdU labeling of ovaries was successfully used to screen for defects in DNA replication during Drosophila oogenesis.

BrdU labeling of Ovaries (and other tissues)

Solutions:
• Grace's insect cell culture medium (BRL 1159-0056)
• 1mg/ml BrdU in Grace's (Boehringer Mannheim cat# 280-879)
• buffer B: 100mM KH2PO4/K2HPO4 pH 6.8, 450mM KCl, 150mM NaCl, 20mM $MgCl_2$)
• α-BrdU mouse monoclonal (Becton Dickinson, cat#347580 (7580))
• DTAF or FITC fluorescent goat o-mouse
• PBT: 1X PBS, 0.1% Triton X-100
• 2N HCl 17.2ml of 11.6 M/100ml final volume (made relatively fresh)
• 100mM Borax (make fresh)
• 20% BSA in PBT (Sigma cat# A-4503)
• 100% donkey serum (or goat) (Sigma cat# D9663) abbreviated as "ds"
• Vectashield (Vector Labs, cat # H-1000)

1. Fatten females w/ males 2-4 days on wet yeast.
2. Dissect in Grace's at room temp. in glass dish and place in 24-well Mesh Plate that is in a Condo Tray with Grace's medium.
3. With large forceps take the 24-well Mesh Plate with ovaries (or other tissue) out of the blue tray and place in a second blue tray with fresh room temp. Grace's with 5µg/ml BrdU (final). Incubate 1hr @ room temp. with gentle shaking, covered with foil.
4. Rinse with Grace's. All washes are on shaker with gentle agitation.
5. Fix for 20 min. with 4:1:1 16% formaldehyde:buffer B:dH_2O (~10.5% formaldehyde final) can substitute PBS for buffer B, make sure PBS is 1x final concentration.
6. Wash w/ PBT 3x 5 min.
7. Acid treat w/ 2N HCl for 30 min.
8. Neutralize for 2 min. w/ 1ml of Borax.
9. Wash w/ PBT 3x 5 min. blotting excess off on paper towel each time.
10. Block w/ PBT/5% donkey/goat serum 30 min. @ room temp.
11. Incubate O/N @4ºC w/ 1/20 α-BrdU mouse monoclonal.
12. Wash w/ PBT 3x 3 min. blotting excess off on paper towel each time.
13. Block w/ PBT/5% donkey serum 2x 15 min. @ room temp.
14. Secondary incubation: Replace PBT/5% ds w/fluorescent goat α-mouse (1/100), in PBT/5% ds.
15. Incubate 2hr @ room temp.
16. Rinse 3x; Wash 3x 5 min. w/ PBT (0.1% Triton).
17. For DAPI staining, use PBT w/ .005% triton or PBS:

• 1ug/ml DAPI final in PBT 10 min. in dark
• Wash 1x 10 min. in PBT

18. With a wide orofice (or cut-off) 200µl pipet tip remove tissue from well and place into a microfuge tube. Aspirate out all PBT and add 2-3 drops of Vectashield. Mount on slide and seal w/ clear nail polish.

The Fly Condo™ Template

Date:_____ Experiment:_____

Notes:

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