
It is important to use essentially cell-free homogenates of Drosophila adults. Standard homogenizers, including relatively expensive ones such as the VirTis Model 45 with micro accessories, fail to give satisfactory homogenization (even after 5 minutes at a maximum speed of 45,000 rpm in the VirTis). The method described here gives homogenates that are essentially cell-free.

A Tri-R teflon tissue homogenizer (920 pestle, 7.82 mm diameter, 0.11-0.15 mm clearance with 330 tube of 10.3 mm OD, capacity, 2 ml; Tri-R Instruments, Inc. 48 Merrick Road, Rockville Centre, New York 11570; about $8.) is the basic item used in this procedure. The shaft of the pestle is coupled by a sleeve of thick-walled rubber tubing to the driveshaft of a Mixco heavy duty stirrer, in which a 1/30 HP motor provides a friction drive at variable speeds up to 1750 rpm (available from Will Corp. and other suppliers; about $75.). In the bottom of the homogenizer tube is placed a small quantity of glass beads, 0.11 - 0.12 mm (Will Corp., Rochester, New York; about $17 per kg). To the tube is then added 2 ml of physiological saline, and finally from 100 to 250 living flies, inactivated by being kept at 40 °C for 5 minutes or more. The flies are homogenized for 45 sec to 1 min while the tube is submerged in a brine bath at -50 °C.

Into a 5 ml disposable plastic syringe (5 cc Plastipak B-D sterile disposable plastic syringes, Cat. No. 8055, Becton, Dickinson and Co., Rutherford, New Jersey; about $12. per 100) place a small quantity (about 100 mg, or a non-compressed volume of about 2 ml in the syringe barrel) of glass wool (Pyrex brand wool, Cat. No. 3950 Filtering Fibre, Corning Glass Works, Corning, New York; about $8 per pound). Attach a plastic Swinney filter unit (Millipore Swinney-13 filter holder, Cat. No. SX00 013 00, Millipore Filter Corp., Bedford, Massachusetts 01730; about $6.50 for 10) and an 18 ga needle, 1 1/2" long. First a disc of Whatman No. 1 filter paper, and then a disc of Miracloth (your local bar supply company, or Miracloth Sales, Chicopee Manufacturing Co., Milltown, New Jersey; about $3. for 50 ft.) cut with a cork borer, should be placed in the filter holder.

The homogenate is transferred to the barrel of the syringe. 2 ml of saline wash is then added to the residue in the homogenizer tube, and the tube is raised onto the rotating pestle 4 or 5 times. The wash is then added to the barrel of the syringe, and the homogenate is expressed through the glass wool by the plunger of the syringe, which should be lightly rimmed with stopcock grease. When all but 1 ml of the homogenate has been pressed through (into, for instance, a small volumetric flask), the syringe barrel is disconnected from the Swinney unit (to prevent rupture of the filter paper and Miracloth discs), the plunger is removed, the syringe is reconnected, and an additional amount of saline (about 5 ml) is added to the syringe. The plunger is depressed again, this time fully compressing the glass wool filter in the barrel. The final volume of the homogenate can then be adjusted with saline; we routinely use 10 ml as a standard volume for subsequent assays.

The glass wool filter retains the glass beads and virtually all the fragments of chitin; the remaining particulate matter that does get through is caught in the Miracloth and filter paper discs. The homogenate is very clean, and contains almost no cells.

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A three per cent solution of NaCl (3 gm. per 100 cc. standard medium prior to solidifying) has effectively controlled a species of mites which parasitizes Drosophila adults. Development time is lengthened due to this severe treatment. The larvae survive satisfactorily, however.

The means of control appears to prevent development of the white nymph stage in the life cycle. These apparently develop from the smaller light brown "protonymph" which attach to the flies. A moderate percentage of "protonymphs" may survive on parent flies and on the medium, but very few appear on their progeny. The organisms which attach to the flies apparently can not reproduce unless the nymphs are able to develop on the medium.

Helpful information was contributed by D. M. Pinkham of Macalaster Scientific Company, Waltham, Massachusetts.