Sample Preparation Protocol SP-11
Drosophila Viability Evaluation

Scope

This protocol describes the method used for investigating the effects of COPAS ESS SHEATH on the viability of *Drosophila*. Larval forms of *Drosophila* were incubated in the COPAS ESS SHEATH. At various time intervals, the organisms were processed on the COPAS *PLUS* instrument and dispensed into microtiter plates. These plates were monitored for viability by trained fly biologists.

Materials

- COPAS ESS SHEATH, 1 liter MINIMUM (Union Biometrica P/N 335-5070-000)
- Fly Wash Solution Containing: 0.2%NaCl and 0.02% TritonX-100, 500 mL MINIMUM (See Sample Preparation Protocol SP-07)
- 24-well microtiter plates filled with fly growth medium containing grape juice and yeast plates, 3 plates MINIMUM
- Day old *Drosophila* larvae on Petri dishes containing fly growth medium

NOTE: Six (6) 100 mm Petri dishes with moderate to marked larval growth yield approximately 150 ml of working larval solution

Procedure

Sample Preparation:

Open Petri dishes. Add approximately 4 ml of fly wash solution to each dish and agitate slightly. Using a small paintbrush, gently brush the organisms from the agar into the fly wash solution.

Decant the larvae from the Petri dishes into 2 or more 15 ml conical tubes.

Pellet the organisms by settling, approximately 4 minutes. Aspirate the supernatant from the pellet and discard.

Re-suspend the pellet in 10 ml of fly wash solution per 15 ml conical.

Mix each tube gently and pellet again by settling. Aspirate the supernatant from the pellet and discard.

Re-suspend the pellet in 10 ml of ESS SHEATH per 15 ml conical.

Mix each tube gently and pellet again by settling. Aspirate the supernatant from the pellet and discard.

Re-suspend the pellet in 10 ml of ESS SHEATH per 15 ml conical.

Mix each tube gently. Each tube should contain CLEAR sheath diluent with monodisperse larval *Drosophila* throughout the mixture. If the mixture is not CLEAR another wash is necessary.

If the larvae is sufficiently washed, dilute the animals with sheath to a final concentration of 150 animals per ml.

COPAS™ Protocol #SP-11

Observe animal viability prior to processing on COPAS instruments. Score approximately 30 larvae for viability.

Score Method:
Example 29 of 30 viable = 29/30
= 96.7 **%VIABLE**

Instrument Processing for COPAS *PLUS (PLUS for example only, other instruments are also intended for use with Drosophila):*
Set up the COPAS *PLUS* as directed in the Operator's Manual.

Add 40 ml of diluted larvae into the sample cup.

Dispense 5 larvae per well into 6 wells of a 24 well microtiter plate (N = 30). Count the total number sorted and score for viability. Place lid on plate. This is the 0 time point. The remaining diluted larvae should be mixed using a standard laboratory rocker.

After three hours, take approximately 40 ml of the remaining preparation and place it into the sample cup.

Dispense 5 larvae per well into 6 wells of a 24 well microtiter plate (N = 30). Count the total number sorted and score for viability. Place lid on plate. This is the 3 hour time point. The remaining diluted larvae should be mixed using a standard laboratory rocker.

After 19 hours take approximately 40 ml of the remaining preparation and place it into the sample cup.

Dispense 5 larvae per well into 6 wells of a 24 well microtiter plate (N = 30). Count the total number sorted and score for viability. Place lid on plate. This is the 19 hour time point.

View and score the 0, 3, and 19 hour plates over the next 72 hours (or until pupation) for number and viability. Monitor the larvae every 24 hours throughout this time and document the number and viability of the larvae on each plate.

Analysis of Data:
Compare the **%VIABLE** of the 0, 3 and 19 hour time points after first being dispensed from the instrument. Also compare the viability of the larva after being dispensed into growth medium for 72 hours or until pupation in 24 increments.

Document all findings.

Questions?
For further information, please contact Union Biometrica, Inc. directly at 617.591.1211 or email your questions to appsupport@unionbio.com