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Sample Preparation Protocol SP-01
Drosophila melanogaster (fruit fly) Embryos

Scope
This protocol is to be used when preparing *Drosophila melanogaster* embryos for use with the COPAS *SELECT* technology platform.

Materials
0.5% PBS/2% Tween-20 Solution (Prepared by user)
COPAS ESS, Embryo Sample Solution (Union Biometrica; P/N 335-5075-000)
840µm, 380µm, and 140µm Sieve Set (Carolina Catalog number: BA-GEO9310)
Wash Bottles
50ml Conical Tubes
Transfer Pipettes
Paintbrush
Dissecting Microscope
Egg Laying Plates with *Drosophila* Embryos

Procedure
Harvesting the Embryos:
The embryos are harvested by adding 10ml of 0.5% PBS/2% Tween-20 directly to the egg laying plates. A small paintbrush is then used to wipe the embryos off the growth medium, so they can be easily transferred into a 50 ml conical tube using a liquid transfer pipette. The embryo solution is then passed through sequential sieves, 840µm and 380µm, that are used to separate debris from the embryos. Once the solution has passed through the sieves, a wash bottle containing 0.5% PBS/2% Tween-20 is used to pass any remaining embryos through the sieves. Finally, a 140µm sieve is used to collect the intact embryos for further processing.

Dechorionation:
The embryos on the 140µm sieve are transferred into a 50% bleach/ 0.5% PBS 1% Tween-20 solution in order to remove the chorions.

NOTE: Check for complete dechorionation by visualizing embryos under a dissecting microscope.

Once the embryos are fully submerged within the bleach solution, they are gently agitated for a MINIMUM of 5 minutes (confirm dechorionation has occurred by viewing embryos under a dissecting microscope). The 140µm sieve is then transferred into a 0.5% PBS/2% Tween-20 solution for exactly 3 minutes in order to minimize embryo clumping. The embryos are then washed off the 140µm sieve into a conical tube using 0.5% PBS/2% Tween-20. Any remaining 0.5% PBS/2% Tween-20 solution is separated from the dechorionated embryos by a 5-minute settling step. After the embryos have settled, the supernatant is removed from the conical tube using a transfer pipette. Only dechorionated embryos should remain pelleted at the bottom of the 50ml conical tube.

NOTE: Once the chorions have been removed, the embryos are very susceptible damage. Care should be exercised while handling the embryos.

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Sample Preparation:
The Embryo Sample Solution (ESS) is added to the 50 ml conical tube containing the embryos until the final concentration of embryos is 0.5 per µl. The volume added to the conical tube depends on the number of embryos originally harvested. While adding the ESS, the embryo concentration should be monitored frequently by extracting a known volume of liquid from the solution and counting how many embryos are present. The ESS has been carefully formulated to reduce clumping between the embryos. The embryo solution is now ready to be transferred to the sample cup on the COPAS *SELECT* for analysis.

Sheath Fluid:
Use COPAS *SELECT* Sheath for rapid embryo sorting applications. The COPAS *SELECT* Sheath must also be added to the high pressure clean out bottle.

NOTE: A stable flow rate between 10-25 embryos / second is recommended for optimal system performance.

Sample Flow Conditions:
A stable sample flow rate is required in order to run the embryos optimally and to avoid system clogging. The system flow rate should be set at 10-25 embryos/sec. A flow rate of 10-25 embryos/sec is achieved by adjusting the sheath pressure and sample pressure.
At a room temperature of 19°C, the sheath pressure should be set between 3.15 to 3.50 psi and the sample pressure should be set between 1.55 to 1.75 psi.

Adjustments to the sample pressure may be required under the following conditions:

1. At the beginning of each run in order to stabilize flow.
2. If the concentration of the embryo solution changes: this may occur when a new sample preparation is added to the sample cup.
3. When the sample solution level drops below 20 ml.

Sort Accuracy:
Once a stable flow rate has been achieved, sort accuracy of the instrument must be checked. The sort accuracy of the instrument is directly affected by the sort delay and sort width parameters. The sort delay is the amount of time from when the laser detects the organism until the sorter valve closes and sorts it. The sort width determines the volume of fluid in which the organism is contained.

The sort parameters should be set at values that produce the highest instrument accuracy. Typically, at a room temperature of 19°C, the sort delay should be set between 14 and 18 ms. The sort width should be set between 12 and 14 ms. The values for these parameters can only be accurately determined while running the embryos on the instrument and dispensing a selected number of embryos onto a test plate for visual confirmation. Adjustments to these parameters may be needed to attain optimal system accuracy.

NOTE: Before each experiment, check for system accuracy by selecting a number of embryos to be dispensed. Visually confirm that the instrument has dispensed the correct number of embryos.

Questions?

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