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Sample Preparation Protocol SP-10
C. elegans (nematode)

Scope
This technique should be used to prepare *C. elegans* prior to processing them on the COPAS instrument.

Materials
M9 Buffer (See Sample Preparation Protocol SP-07)
0101% Triton X-100
180µm nylon mesh (Millipore Corp. number NY8H04700)
10 µL eppendorf pipette

Procedure

NOTE: For final test the final concentration of organisms should be 1/µL. For normal processing of the instrument, concentrations of organisms should be 0.5 to 2.0 / µL.

Wash animals off agar medium using M9 buffer with 0.01% Triton X-100. In the event of heavy contamination of the culture, or if liquid medium is used, the sample should be passed through a 180µm nylon mesh to remove larger clumps of eggs and debris.

NOTE: For most applications 2 agar plates with moderately heavy growth are enough for approximately 100 mL of final worm prep at a concentration of 1 organism / µL.

If the preparation is excessively debris ridden perform a sucrose float technique (See Sample Preparation Protocol SP-04 for Sucrose Float Technique details).

For the FINAL TEST PROCEDURE on the COPAS *BIOSORT* instrument, 40 ml per instrument is necessary. The final concentration of organisms should be 1 / uL, and the percentage of adults should be no more than 25%. Standard N2 worms are recommended, but any plate with mostly viable worms can be used.

To determine the concentration of organisms from the washed plates, adjust a 10 µL eppendorf pipette to aspirate 5 µL.

Mix the worm suspension, and aspirate 5 uL of sample. Expel the 5 µL of sample onto a microscope slide. Repeat mixing and expelling steps two more times. (Total: 3 drops on slide, each containing 5 µL of worm suspension)

Using 4X or alternate objective, count the number of worms and eggs present in the 15 µL (N). Calculate the concentration of animals/uL using the following formula:

ANIMALS/µL = N / 15

Adjust the concentration of animals to approximately 1.0 per uL by adding M9 diluent.