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COPAS *BIOSORT* Control Protocol CB-05 Using Low-Level Fluorescent Particles

Scope

This protocol is intended for use in combination with COPAS Operator's Manuals to aid users in the use of low-level fluorescence control particles for experiments that require increased fluorescence sensitivity.

COPAS low-level fluorescence control particles are specially manufactured to simulate a dilute organism population. It is recommended that these particles be processed prior to any experiment requiring increased sensitivity in fluorescence. The guidelines for instrument setup are the manufacturers suggestions. It should be noted that each experiment after the initial setup with this particle may require additional changes in gains, PMT voltages, etc. for enhanced viewing and sorting of the population desired.

- NOTE: COPAS low-level fluorescence control particles settle upon standing. Particles must be mixed prior to each use.
- NOTE: Do not put COPAS low-level fluorescence control particles into the secondary sample container. Foaming will occur.

Procedure

Set up COPAS instrument as directed in the Operator's Manual.

Mix COPAS low-level fluorescence control particles by inversion 4 times. Do not shake and avoid any excess agitation as this may cause bubbles in the mixture.

Place 20 ml (MINIMUM) of COPAS low-level fluorescence control particles into the primary sample cup. On the COPAS software main screen, select the TOOLS Menu and access the Adjust Gain Values mode. Change the settings to read the following:

EXT FULL SCALE	256
EXT SIGNAL GAIN	40
EXT INTEGRAL GAIN	240
EXT THRESHOLD	40
FLU FULL SCALE	256
FLU SIGNAL GAIN	40
FLU INTEGRAL GAIN	240
TOF FULL SCALE	256
TOF MINIMUM	10
PMT	550*

*All PMT'S are different. The above setting is suggested and may have to be adjusted for your instrument.

Document the instrument pressures.



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Process the particles until 1000 events have been collected.

Observe the COPAS software main screen. The data may be stored and analyzed for peak, mean, and C.V. using WIN MDI statistical software.

At the above settings, low-level fluorescence control particles have the following *approximate* mean channel number and C.V.:

Parameter	Mean Channel	C.V.
TOF	15	9.0
EXT	40	13.0
FLU	25	15.0

Cleaning Procedure:

After using the low-level fluorescence control particles, detach the sample and pressure hoses (sample first). Open the primary sample cup and rinse with distilled water.

Aspirate off the distilled water and rinse again with distilled water.

Cap the primary sample cup, attach the pressure hose only, and process the distilled water for approximately three (3) minutes.

Then initiate the CLEAN sequence.

After cleaning, open the primary sample cup and aspirate off the distilled water.

The instrument is now ready for use.

Questions?

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