COPAS XL Control Protocol CXL-02
Sort Optimization Protocol

Scope
This protocol is intended for optimization of the SORT SETTINGS for the COPAS *XL* instrument.

Introduction
The analysis and sorting of an organism is a function of the sheath pressure setting. This pressure determines the velocity of the organism. The speed in meters/second is dependent on the diameter of the flow cell and the pressure. The difference in time between the moment of analysis of the organism and the sort command is called “sort delay”. The sort delay time is indicated in milliseconds. The period of time that the sort valve is closed is called sort width. The sort width determines the volume of fluid the selected organism is contained in. In order to minimize and/or optimize the settings, the following procedure can be followed.

Introduction to Methods
The COPAS *XL* instrument is intended for analyzing and sorting of *Drosophila* (fruit fly) and *D. rerio* (zebrafish) larvae. For *rapid* and *precise* sorting of a selected population of animals the following 5 criteria must be met:

1. The sample must be cleaned of debris.
2. After the initial setup of the instrument, ambient temperature must not fluctuate more than 1.5ºC.
3. COINCIDENCE CHECK must be ON.
4. The sort delay and sort region, in that order, must be set accurately.
5. The instrument must be *cleaned thoroughly daily* and in between processing of different sample types.

Methods
Refer to the COPAS Operator’s Manual for a description of the COPAS software main screen. Set up the COPAS *XL* as recommended in the Operators Manual with the following exceptions:

Reagents:
For *Drosophila* applications, use COPAS ESS SHEATH (P/N 335-5070-000) and COPAS ESS Embryo Sample Solution (P/N 335-5075-000). The sample must be diluted with the appropriate sheath prior to processing.

For *D. rerio* applications use COPAS Z SHEATH (P/N 370-5070-000). The sample must be diluted with the appropriate sheath prior to processing.

Instrument Pressures:
Set the COPAS *XL* instrument pressures as follows:

SHEATH PRESSURE	4.0 to 5.0 PSI
SAMPLE *VACUUM**	-0.15 to –0.3 PSI
SORTER PRESSURE	11 to 12 PSI
CLEAN PRESSURE	12 to 13 PSI

*NOTE: The COPAS *XL* sample is regulated by vacuum, not pressure. To increase sample flow, turn sample valve counterclockwise. Conversely, to decrease sample turn sample valve clockwise

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Sample Dilution:
For *Drosophila* applications, a concentration of 50 to 100 embryos or larvae/ml is recommended.

For *D. rerio* applications, a concentration of 15 to 20 embryos or larvae/ml is recommended.

Start the instrument and acquire sample.

If sample is not flowing, turn down the sample vacuum until organism flow begins and data is visible on the dotplot. Slowly increase the sample vacuum until no flow is seen. Then decrease the sample vacuum slowly until flow is started and stable. The sample pressure should be slightly over the threshold of the NO FLOW sample vacuum for the narrowest sample stream.

Adjust GAIN, DELAY, AND WIDTH settings as follows:

	Drosophila	*D. rerio*
TOF Min Chan	10	10
EXT Integral Gain	15	15
EXT Threshold	80	80
EXT Signal Gain	15	15
FLU1 Integral Gain	100	50
FLU1 Signal Gain	100	50
FLU2 Integral Gain	100	50
FLU2 Signal Gain	100	50
EXT FULL SCALE	2048	2048
TOF FULL SCALE	32768	16384
FLU1 FULL SCALE	1024	512
FLU1 PMT	500	600
FLU2 FULL SCALE	1024	512
FLU2 PMT	600	700
DELAY	50	62
WIDTH	35	35

Set the sort width to 35 (MINIMUM). The approximate size of the drop at these settings is between 35 and 50uL. Therefore, 7 to 10 larvae can be dispensed into each well of a 96 well microtiter plate.

*NOTE: When dispensing zebrafish embryos or larvae into wells, pre-fill each well with 150µL of *XL* sheath to cushion the embryo.

Select a region for sorting. Turn COINCIDENCE CHECK ON.

Using the FILL PLATE MODE, sort 1 organism per well into 12 wells of a microtiter plate. Review the plate by microscope to determine if the sort delay is set correctly. Repeat if necessary, changing the delay, until 12 single organisms are collected.

The instrument is now ready for loading multiple plates with multiple animals as needed.

NOTE: The instrument may be left unattended at this point. If large sort numbers are selected, the instrument should be checked periodically.

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Instrument Clean Up

The COPAS XL must be rinsed thoroughly with DISTILLED WATER at the end of each day, especially if using ESS sheath and embryo sample solution in the instrument.

Replace the 10 liter sheath container with the extra 4 liter sheath container containing distilled water. Rinse the sample cup out twice with distilled water. Fill the sample cup with distilled water and cap tightly. *Process sheath and sample for approximately 3 minutes to rinse out the lines.* If using the secondary sample container it must also be cleaned with distilled water and water must be processed through the sample lines.

If using *XL* sheath reagent, the sample cup should be rinsed out twice with clean distilled water or *XL* sheath reagent. Place clean distilled water or *XL* sheath in the sample cup, cap tightly and process sample and sheath for at least three minutes to clean out the sample lines.

Questions?

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