

# COPAS BIOSORT Control Protocol CB-02

## Sort Optimization Protocol

### Scope

This protocol is intended for optimization of the SORT SETTINGS for the COPAS BIOSORT 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 amount of time, in milliseconds, from the moment of analysis of the object to the sort command is called “sort delay”. The amount of time, in milliseconds, that the sort valve is closed is called sort width. The sort width determines the COINCIDENCE CHECK and the volume of fluid the selected object is contained in. Sort Width and Coincidence Check time have an immediate effect on the sort yield (effective sort recovery). Minimizing the Sort Width time improves the yield by reducing coincidence. If, however, the Sort Width time is set too low, contamination of the sorted population by extra-sorted events or missed events can occur. In order to minimize and/or optimize the settings, the following procedure can be followed.

### Materials

Control Sample  
Microscope Slide  
Microtiter plate

### Procedure

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

Run a control (N2) sample and select the large organisms by setting a gate on a TOF versus EXT dot plot. Set the sort width parameter to 2.5 (this is the MINIMUM value tested). Select positions A1, A3 and A5 on the 96 well plate layout. Set the number of events per well to 10.

Press “Fill Plate” and count the sorted events dispensed on the cover of a microtiter plate. Increase or decrease the Sort Delay (preset to 8.0) by 0.1 milliseconds each time and control the amount per spot on the microtiter plate cover. Repeat until the optimal result (10 events in each spot) is achieved.

NOTE: The sort width must be less than the sort delay.

NOTE: The sort delay setting is optimal for the current sheath pressure. If the sheath pressure is changed, the test must be repeated.

### Questions?

**For further information, please contact Union Biometrica, Inc. directly at 617.591.1211 or email your questions to [appsupport@unionbio.com](mailto:appsupport@unionbio.com)**