

# COPAS in Bead Applications Control Protocol CPB-02

## Sort Optimization Protocol

### Scope

The intended use of this protocol is to supplement the COPAS Operator's Manual with additional instrument guidelines for use in bead applications.

### Introduction to Methods

For *rapid* and *precise* sorting of single beads the following 5 criteria must be met:

1. The sample *must be* prepared in sheath diluent before processing on the dispenser.
2. After the initial setup of the instrument, ambient temperature must not fluctuate more than 1.5°C. (See temperature graphs in OPERATOR'S MANUAL.)
3. A stable sample stream must be attained and COINCIDENCE CHECK 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.

### Attaining a Stable Sample Stream

Set up the COPAS instrument as outlined in the Operator's Manual with the following exceptions:

1. Set the sheath pressure between 3 and 4 psi.
2. Set the clean pressure to 7 psi.
3. Set the sample pressure to 1.5 psi or lower until the sample begins to flow.

NOTE: The sample pressure is less than the sheath pressure in this instrument.

Add a prepared, clean, sample to the primary sample cup.

NOTE: For precise selection of beads of a specific size from a mixed population, a concentration of 60 beads / ml is recommended.

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

## Setting Accurate Sort Delay and Sort Region

Optimize the instrument gains for sorting and select a region for sorting. Turn COINCIDENCE CHECK ON.

Preliminary GAIN settings:

TOF Min Chan	10
Integral Gain	50
Threshold	100
Signal Gain	80
Max Extinction	2048
Max TOF	2048

Set the sort width of the instrument to 6 (MINIMUM). Set the sort delay to 13.

Using the FILL PLATE MODE, sort 1 bead per well into 12 wells of a microtiter plate or lid. Review the plate by microscope to determine if the sort delay is set correctly.

Repeat if necessary, changing the delay, until at least 11 of 12 single beads are collected. The sort width can now be made higher if necessary.

Using the FILL PLATE MODE, sort 1 bead per well into 12 wells of a microtiter plate or lid. Review the plate to determine if this is the population of choice.

If you have not selected the population of choice, adjust the sort region and repeat until the region is determined.

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

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

## Instrument Clean Up

*The COPAS instrument must be cleaned thoroughly with ethanol at the end of each day*

Place approximately 40 ml of ethanol in the sample cup. Process SAMPLE ONLY for APPROXIMATELY 5 minutes or until the primary sample cup is almost empty.

NOTE: A warning will appear on the COPAS screen, disregard and select OK.

Rinse the primary sample cup with ethanol. Aspirate the ethanol and discard.

## 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)**