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COPAS in Bead Applications Control Protocol CPB-01 Sort Optimization Protocol using UV Laser

Scope

The intended use of this protocol is to supplement the COPAS Operator's Manual with additional instrument guidelines for use with the COPAS instrument with UV Laser for Combinatorial Chemistry applications.

Introduction to Methods

For *rapid* and *precise* sorting of single beads the following 4 criteria must be met:

1. After the initial setup of the instrument, ambient temperature must not fluctuate more than 1.5ºC. (See temperature graphs in Operator's Manual.)
2. A stable sample stream must be attained and COINCIDENCE CHECK ON.
3. The sort delay and sort region, in that order, must be set accurately.
4. The instrument must be *cleaned thoroughly daily* and in between processing of different sample types.

Methods

Set up the COPAS instrument as directed in the Operators Manual with the following exceptions:

1. Set the sheath pressure of the COPAS PLUS between 5 and 6 psi.
2. Set the sample pressure of the COPAS PLUS to 2.5 psi.

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 30 beads / ml is recommended.

Start the instrument and acquire sample. If sample is not flowing turn up the sample pressure until bead flow begins and data is seen on the dotplot. Slowly decrease the sample pressure until no flow is observed. Then increase the sample pressure slowly until flow has 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
EXT Integral Gain	30
EXT Threshold	60
EXT Signal Gain	40
FLU Integral Gain	100
FLU Signal Gain	100
EXT FULL SCALE	1024
TOF FULL SCALE	1024
FLU FULL SCALE	512
FLU PMT	500

Set the sort width of the instrument to 20 (MINIMUM). Set the sort delay to 23. 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 40mL 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, acknowledge and select OK.

Rinse the primary sample cup with distilled water. Aspirate the distilled water and discard.

Fill the primary sample cup with distilled water. Process sample and sheath for approximately 2 minutes to clean the sample lines. Process SHEATH ONLY for another 10 minutes to clean before instrument shutdown.

Questions?

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