



STarSystem Control Microsphere (Con1 and Con2) Overview.

The Control microspheres (L100-CON1 and L100-CON2) are used on the IS version Luminex 100 system only (not on 1.7 version Luminex instruments) to verify calibration and the optical integrity of the system.

xMAP Classification Control Microspheres (CON1) are used to verify the calibration and optical integrity for both the classification channels and doublet discrimination channel for the Luminex® 100™ IS system.

xMAP Reporter Control Microspheres (CON2) are used to verify the calibration and optical integrity for the reporter channel for the Luminex 100 IS system.

STarStation Version 1.1 software does not currently possess the functionality to perform CON1 or CON2 calibration validation steps. STarStation Version 2.0, which is currently in development, will allow Con1 and Con2 calibration validation steps to be performed.

Classification Control Beads (CON1) are used to check the microsphere classification efficiency. The CON1 reagent is a mixture of microspheres with five different color-codes used to check that the bead classification channel is functioning correctly. The microspheres are selected to be on the periphery of the bead map, the microsphere colour codes used are 6,21,73,81 and 96.

Reporter Control microspheres (CON2) are a mixture containing roughly equal concentrations of 4 microspheres with different concentrations of proprietary reporter fluorophores. CON2 beads are used to check reporter channel response over the dynamic range of the cytometer. The CON2 beads have no classifier dye but have four levels of RP1 proprietary dye giving a 3 decade dynamic range and sensitivity check. These beads are not strictly a 4-plex since they cannot be individually identified or "de-multiplexed" by gating.

Examples of the target data sheets for CON1 and CON2 microspheres are shown below (Figures 1 and 2). Examples of data displays indicating how CON1 and CON2 microspheres are detected via the STarSystem detectors are shown in Figures 3 and 4 below.

Certificate of Quality
xMAP™ Classification Control Microspheres, CON1

Product Number	L100-CON1		
Lot Number	A3635		
Expiration Date	27 FEB 2004		
	<u>Parameter A1</u>	<u>Parameter B1</u>	<u>Parameter C1</u>
	<u>Target Value</u>	<u>Target Value</u>	<u>Target Value</u>
Reference 01	10145	765	220
Reference 02	9501	73.3	64.8
Reference 03	10036	23159	5636
Reference 04	8553	2522	17738
Reference 05	9048	230	5748

Figure 1.

Certificate of Quality
xMAP™ Reporter Control Microspheres, CON2

Product Number	L100-CON2
Lot Number	A3689
Expiration Date	27 FEB 2004
	<u>Parameter A1</u>
	<u>Target Value</u>
Reference 01	29.1
Reference 02	197
Reference 03	1910
Reference 04	17429

Figure 2.

The reporter (RP1) channel is the fluorescence channel used for assay quantitation R-phycoerythrin (R-PE) is the primary reporter molecule used in Luminex assays. A series of beads dyed with varying intensities of a fluorochrome spectrally matched to R-phycoerythrin are used for this procedure. Each of the reporter beads has been assigned a specific intensity value corresponding to the number of fluorescent R-PE molecules. These units of fluorescent measure are known as molecules of equivalent soluble fluorescence (MESF). MESF units allow direct correlation of instrument performance to a typical assay using R-PE as the indicator molecule.

The available range of channels on the STarSystem Cytometer is 4.5 log amp decades or 32,767 relative linear channels.

Always run system controls after calibration and as needed for troubleshooting.

To minimize carryover ACS recommends a wash with distilled water or sheath fluid between instrument calibration and instrument control steps.

The target values from the control reagent data sheets are entered in StarStation version 2.0 via the reagent manager.

Control verification is carried out in two phases. The first phase of control verification verifies the side-

scatter, classification one, and classification two channels. The second phase of control verification control verifies the reporter channel.

What procedures should I follow if Calibration and/or Controls fail?

1. Verify that the correct Lot numbers and Target Values are selected.
2. Vortex the microsphere vials.
3. Allow the microspheres to reach room temperature before performing calibration/validation.
4. Verify that the correct wells have been selected if using the XY Platform.
5. Remove the sample probe and sonicate the narrow end for 2-3 minutes.
6. Using a syringe, flush the sample probe with distilled water from the narrow end out through the larger end.
7. Readjust the sample probe height upon replacement in the arm.
8. Complete 3 Backflush, 3 Drain, 2 Alcohol Flush and 3 Washes with water.
9. Repeat calibration. Verify that the pressure readings are between 6 and 9 psi and the Events/Second rate reads around 200 (note the event rate indicator is not available in STarStation version 1.1).

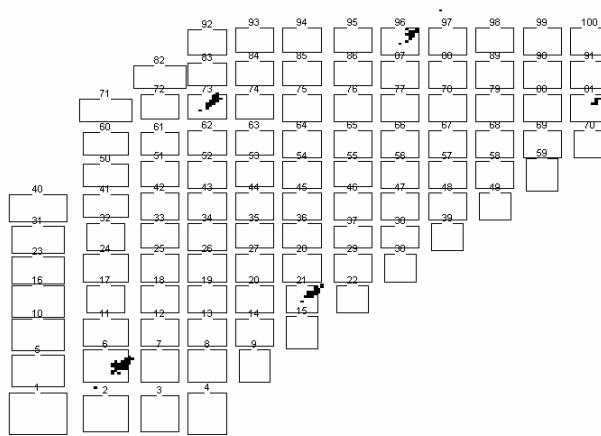


Figure3. CON1 Classification Microspheres on the100 region bead map (STarStation Classifier Plot).

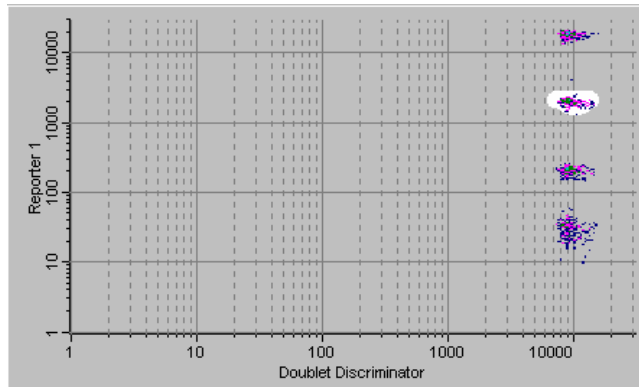


Figure4. CON2 Reporter Control Microspheres (Luminex IS Software Simplex Mode Plot).