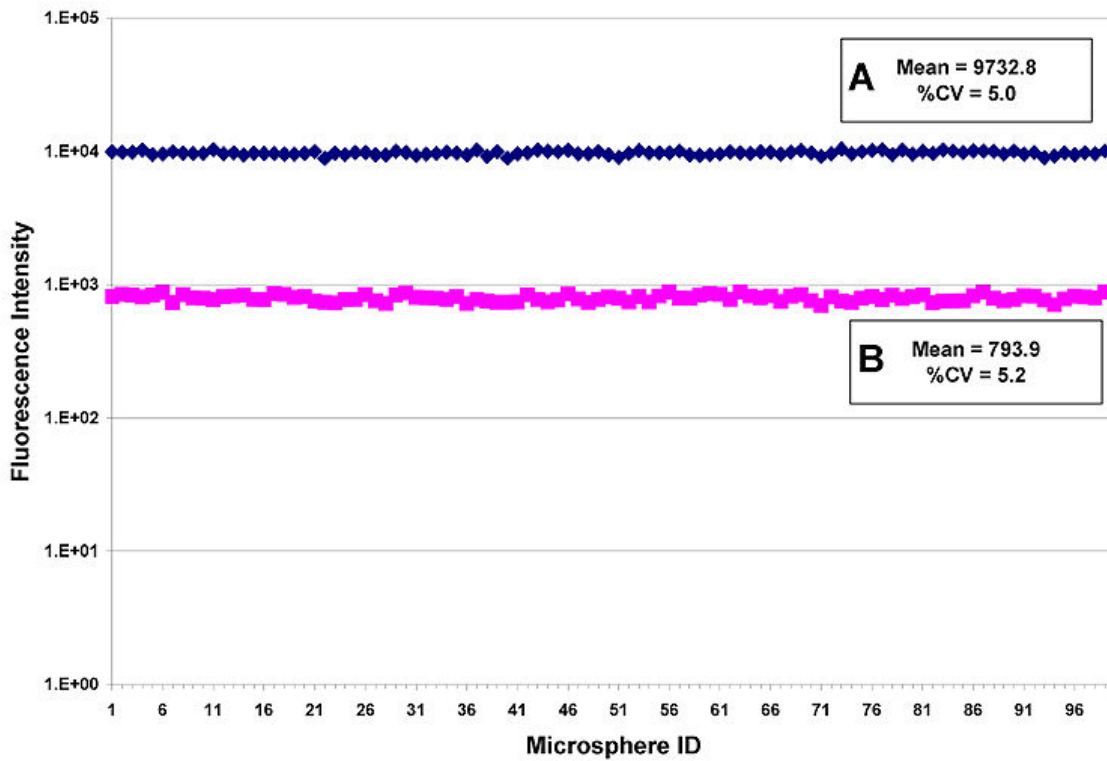


## Are certain microsphere sets better than others?

We are often asked if some of our xMAP microsphere sets are better suited than others for developing assays and with 100 sets from which to choose, it may seem reasonable that some would outperform others. However, the same progenitor microsphere lot is used to produce all of the spectrally distinct sets. This means that the microsphere surface – upon which the coupling and subsequent reactions occur – is identical amongst all 100 microsphere sets. The only difference is the amount of the two classification dyes embedded within the microspheres. To demonstrate microsphere equality, we conducted a study to assess the performance of a model assay across all 100 microsphere sets.

Biotinylated BSA was covalently coupled to each of the 100 xMAP microsphere sets at two coupling concentrations (ten and one microgram biotinylated BSA per ten million microspheres in our standard 500 microliter two-step EDC coupling procedure), reacted with streptavidin-conjugated R-phycoerythrin and analyzed with the Luminex100/200 system. Results are shown in the accompanying figure. The fluorescence value for each microsphere set is the average of replicate assays. Background fluorescence values associated with blank microspheres ranged from 1 to 79 showing the generally increasing values consistent with the higher microsphere sets containing increasing amounts of classification dyes. Because the background values are low compared to reporter fluorescence, they have not been subtracted. As illustrated in the graph, reporter fluorescence intensity is remarkably uniform across all microsphere sets for both coupling concentrations (A = 10 mg/coupling, B = 1 mg/coupling). The overall difference among assay points, as measured by coefficient of variation (%CV), is only 5%. Given that the variability encompassed by this measurement includes 200 separate microsphere couplings, several hundred replicate assays and any residual error introduced by pipetting and other laboratory procedures, it seems safe to assume that your assay will perform equally well on any one of the 100 xMAP microsphere sets.



## In what order should I select microsphere sets for my assay?

If you are not sure which bead regions to order, may we suggest the following table as a guide. Select beads starting from the upper left, reading across to the end of the row, and then down to the next row. Remember all of the bead sets are derived from the same progenitor materials and differ only in the amounts of classifications dyes present. The higher the microsphere number, the more dye present. Higher numbered microsphere sets may have proportionally higher background fluorescence and may be more susceptible to the cumulative effects of exposure to ambient light.

033	034	035	036	037	038	042	043	044	045
046	047	051	052	053	054	055	056	061	062
063	064	065	066	072	073	074	075	076	077
017	018	019	020	021	024	025	026	027	028
029	032	041	011	012	013	014	006	007	008
083	084	085	086	087	088	089	090	048	057
058	067	068	069	078	079	080	002	003	004
009	015	022	030	039	049	050	059	060	070
071	081	082	001	005	010	016	023	031	040
091	092	093	094	095	096	097	098	099	100