

Genisphere protocol for amplifying signal using Anti-PE Biotin UltraAmp products with Luminex bead based kits.

Version 1.3 (06-22-06)

1. Perform the standard assay as directed by the kit product insert, ending at the point in the assay where the beads are bound with the initial streptavidin-phycoerythrin (SA-PE) label.
2. The beads should be washed 3X after the SA-PE step with the kit recommended Wash Buffer using a standard filter plate on a vacuum manifold.
3. Add 50-100ng (5-10uL) of anti-PE biotinylated dendrimer to each well diluted in 50-100uL of Binding Buffer I (product code ABB005). Incubate with constant gentle agitation on an orbital shaker at 300RPM for 30-60 minutes at 37°C.
4. Wash the beads 4X with room temperature 1X PBS with 0.02% Tween.
5. Add another aliquot of SA-PE as was used in the standard Luminex assay. Alternatively, SA-PE from Prozyme diluted to 1-2ng/uL in 1X PBS with 0.02% Tween may provide additional signal enhancement. Incubate for 10-15 minutes at 37° C.
6. Wash the beads 4X with RT 1X PBS with 0.02% Tween.
7. Add 100-125uL of 1X PBS with 0.02% Tween to each well. Vortex prior to acquiring data on the BioPlex instrument.