

Method for Multiplex Assay (For Human and Mouse Assays)

A 96 well filter plate (Millipore) was wetted with 25ul of Assay Buffer (Upstate), vacuumed and 50ul of standard/sample/control was added to the appropriate wells. Then 25ul of a mixture containing requested cytokines (1:20 dilution) that have been conjugated to beads is added. The plate is then placed on a shaker, at 4° overnight.

The plate was then vacuumed, 50ul of Assay Buffer added to each well, the plate was vortexed, vacuumed again, and 75ul of Assay Buffer added to each well. Then 25ul of a mixture containing requested cytokines that have biotin labeled (1:20 dilution) is added to each well. The plate is then placed on a shaker at room temperature for 90 minutes. Then 25ul of Phycoerythrin (1:25 dilution) is added to each well and the plate is placed back on the shaker for 30 minutes. Stop solution is then added at 25ul per well and the plate placed back on the shaker for 5 minutes. The plate is then vacuumed and 125ul of Assay Buffer is added to each well and the plate is placed on the shaker for 1 minute and then vortexed.

The plate is then read utilizing Luminex technology and IS software and the final concentrations are calculated using Upstate multiplexing software.