

Method for Sandwich ELISA (For Human and Mouse Assays)

Human/Mouse XXXXX was measured by two-antibody ELISA using biotin-streptavidin-peroxidase detection. Polystyrene plates (Maxisorb; Nunc) were coated with capture antibody in PBS overnight at 25°C. The plates were washed 4 times with 50mM Tris, 0.2% Tween-20, pH 7.0-7.5 and then blocked for 90 minutes at 25°C with assay buffer (PBS containing 4% BSA (Sigma) and 0.01% Thimerosal, pH 7.2-7.4). The plates were washed 4 times and 50µl assay buffer was added to each along with 50µl of sample or standard prepared in assay buffer and incubated at 37°C for 2h. The plates were washed 4 times and 100µl of biotinylated detecting antibody in assay buffer was added and incubated for 1h at 25°C. After washing the plate 4 times streptavidin-peroxidase polymer in casein buffer (RDI) was added and incubated at 25°C for 30min. The plate was washed 4 times and 100µl of commercially prepared substrate (TMB; Neogen) was added and incubated at 25°C for approximately 10-30 min. The reaction was stopped with 100µl 2N HCl and the A450 (minus A650) was read on a microplate reader (Molecular Dynamics). A curve was fit to the standards using a computer program (SoftPro; Molecular Dynamics) and cytokine concentration in each sample was calculated from the standard curve equation. The range of this assay is XXX to XXX pg/ml. Levels below this range should be interpreted as “Low” (below the lower detection limit). Because of the shape of the standard curve, negative values are occasionally calculated for some samples. These should also be interpreted as “undetectable.” Values above the range are calculated by extrapolation and thus may not be accurate. We have marked those samples that are above or below the range in the “In-range” column of the results as “High”.