Supplementary Material

Measurement of IgG in cell culture supernatants

Experimental

The microplate was precoated overnight at room temperature (RT) with 300 µL of antibody against the Fc portion of mouse IgG (670291, MP Biomedicals, Irvine, CA, USA), at a concentration of 300 µg/L in coating buffer (15 mM Na₂CO₃, 35 mM NaHCO₃ and 3 mM NaN₃). The plate was washed and 200 µL of cell supernatant or purified antibody at several dilutions from 1:1,000 to 1:300,000 were added. Nonspecific IgG from murine serum (Sigma I5381) was pipetted in parallel as standard in triplicate at concentrations of 0.1, 0.3, 1, 3, 10, 30, 100, 300 and 1000 μg/L, respectively, and the plate was incubated overnight. After washing, the plate was blocked with 300 µL of 1% casein in PBS for 30 min at RT. After washing the plate, 200 µL of horseradish peroxidase labeled anti mouse IgG, developed in horse (1:50,000 in PBS, PI-2000, Vector, Burlingame, CA, USA), was incubated for 1 h. After another washing step, 200 µL of peroxidase substrate (240 µL of TMB stock solution and 80 µL of 1% H₂O₂ in 20 mL of citrate buffer) was added and the plate was stopped after 4.0 min by adding 100 µL of stop solution (5% sulfuric acid) and finally the plate was read out at 450 nm. For fitting the curve, a 4-parameter logistic function was used.

$$y = d - \frac{(d-a)}{1 + (x/c)^b}$$

x = concentration of mouse IgG [μ g/L], y = absorbance at 450 nm, a = minimum absorbance (lower asymptote), b = slope parameter, c = curve midpoint [μ g/L], d = upper asymptote

Results

Characteristic calibration curves were derived from mouse IgG standard solution and medians and standard deviations are plotted (Fig. A). Typically, concentrations down to 1 μ g/L IgG, corresponding to an absorbance of about 0.03, could be measured reliably. Variation coefficients of antibody samples measured at different dilutions were in the range of 1% to 5%.

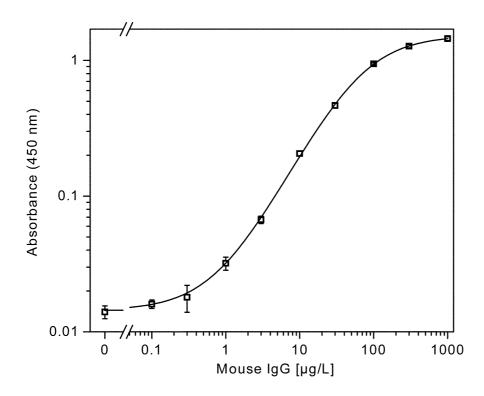


Fig. A Calibration curve of mouse IgG assay. Medians are plotted and standard deviations as error bars, respectively (n = 3).