Elisa assay

Graph Calibration curve The calibration curve is found to be good in terms of its statistical significance, as having R2 value of 0. 94. And can be used as standard curve for Rabbit Ig G. There is slightly deflection at concentration of 2. 0 this might be due to pipetting error as points before and after of it follows in line. It is important to use multichannel pipette to avoid any manual error at a time of filling reagents to microtiter plates. Similarly, to avoid error like at concentration of 2. 0 there must be at least 3 replicates which make data more meaning full and realistic in terms of statistic.

Graph 2: Monoclonal mouse anti-rabbit Ig G

In this graph various dilution of mouse anti rabbit Ig G antibodies were used to determined concentration of rabbit Ig G. the most effective dilution will be between 1/4000 to 1/8000, as in rest of the cases, at lower concentration of Ig G there is no linearity with increasing concentration of rabbit IgG (like in case of 1/2000). At1/2000 dilution concentration of mouse anti-rabbit Ig G is too high compared to rabbit IgG and hence there is no linier relation. Similarly at more than 1/8000 dilution concentration of anti rabbit IgG becomes to less compared to concentration of rabbit Ig G. hence there will not be any anti rabbit Ig G available to bind with rabbit Ig G at higher concentration. In given scenario the optimum dilution for mouse anti- rabbit IgG will be 1/6000 which gives linear correlation with increase in concentration of rabbit IgG.

Graph 3: Polyclonal Goat anti-rabbit Ig G:

Here in case of goat anti rabbit Ig G the over all binding and absorption profiles restricts it use for estimation of rabbit IgG. There are two different scenario 1) In case of lower dilution (1/2000) there is lots of non specific binding resulted in to very high absorption at lower concentration of rabbit Ig

G and get saturated at slightly higher concentrations of rabbit Ig G.

2) In case of higher dilution (1/4000 and above) there is very less binding due to non specificity of polyclonal anti body. Due to less binding there is no significant increase in absorption even at higher concentration of rabbit Ig G. In conclusion polyclonal Goat anti rabbit Ig G is not very useful for determination of titer of rabbit Ig G due to its nonspecific binding and specific activity.

Based on above three graphs it can be concluding that;

1) Calibration curve has to be performed again In triplicates to get statically significance data.

2) There should be use of multichannel pipette to avoid any sample to sample variation

3) Mouse monoclonal anti-rabbit Ig G is better choice at dilution of 1/6000 to determined rabbit Ig G titer up to 2000ng/ml

4) Goat polyclonal anti rabbit Ig G will not be a good choice for this estimation due to higher non specific binding and can be avoided.

5) But at extremely low concentration of rabbit Ig G (less than 300ng/ml) goat polyclonal anti rabbit IgG can be employed by diluting it to (1/4000) as its give significant absorption. But one can not 100% relay on this results.