



Steps required for removing interference with ELISA-based approach

We are developing a human monoclonal antibody therapeutic and are aware that soluble target present within samples may interfere with ELISA-based bioanalytical approaches. What steps can be taken to minimize or remove target interference?

Two popular ELISA design strategies for measuring levels of monoclonal antibody therapeutics in a human matrix include antigen capture or anti-idiotype capture approaches. Both are subject to target interference involving competition by the endogenous target with the binding of the monoclonal antibody therapeutic to the immobilized capture reagent.

To establish the sensitivity of a procedure to target interference within the prototype assay, we must conduct an experiment in which soluble target is spiked into (over a range of concentrations) samples containing known spiked-in amounts of monoclonal antibody therapeutic and then is followed by an equilibration period and analysis. If the anticipated concentrations of target within the study samples are below those concentrations that cause assay interference, then the prototype assay is likely to be fit for its intended purpose, without further modification. In contrast, if the anticipated concentrations of target in the study samples are above those known to cause target interference, then any study results would have to be interpreted accordingly, or the assay should be modified in order to minimize, eliminate or quantify the interference.

The following approaches can be assessed:

Dissociation

The monoclonal antibody therapeutic and soluble target are likely to be equilibrated in the form of a complex within the study samples. In order for the antibody-target complex to bind to the ELISA capture reagent, it has to dissociate first. This can be achieved by the addition of a dissociation solution to the sample, followed by dilution with a neutralization solution, directly to the ELISA plate that is coated with the capture reagent. This allows the

now free monoclonal antibody therapeutic to bind to the capture reagent and soluble target on equal terms, and can provide an assay with greater, but not complete, resistance to the to target interference.

Denaturation

Occasionally, it is possible to identify a dissociation or denaturation solution that can be added to the sample that dissociates the target, and prevents the soluble target, but not the monoclonal antibody therapeutic, from returning to an active conformation upon neutralization. In these cases, target interference can be eliminated.

Anti-idiotype design (bound and total assays)

Development of anti-idiotype antibodies that recognize the complex of the therapeutic monoclonal antibody and the target will assist in the development of a method to assess the 'complexed' therapeutic monoclonal antibody. This assay format can be modified to determine 'total' therapeutic monoclonal antibody by the addition of excess soluble target to the sample, so that all the antibody molecules become bound to target.

Target assay

Co-development of a method to determine target concentrations can identify the samples in which target interference is likely to influence the measured concentrations of therapeutic monoclonal antibody. These assays are also useful for pharmacodynamic (PD) assessments.

No single approach can be applied to all monoclonal antibody therapeutics. Investment in the development of a collaborative approach to assay development well ahead of the intended clinical study start date will facilitate the provision of a fit-for-purpose assay on time.