

Assay Development: Testing Non-Specific Binding for Antibody Qualification

- Unique dotLab® ability to test multiple parameters in a single assay • Immediate identification of cross-reactive species
- Fast assay development with optimal assay conditions

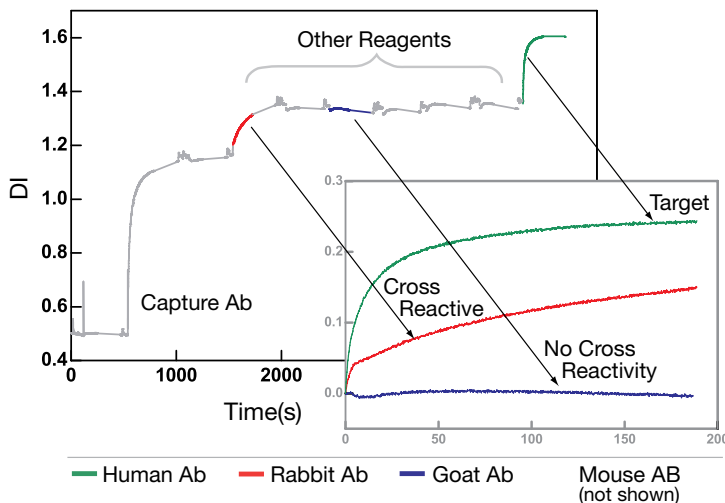
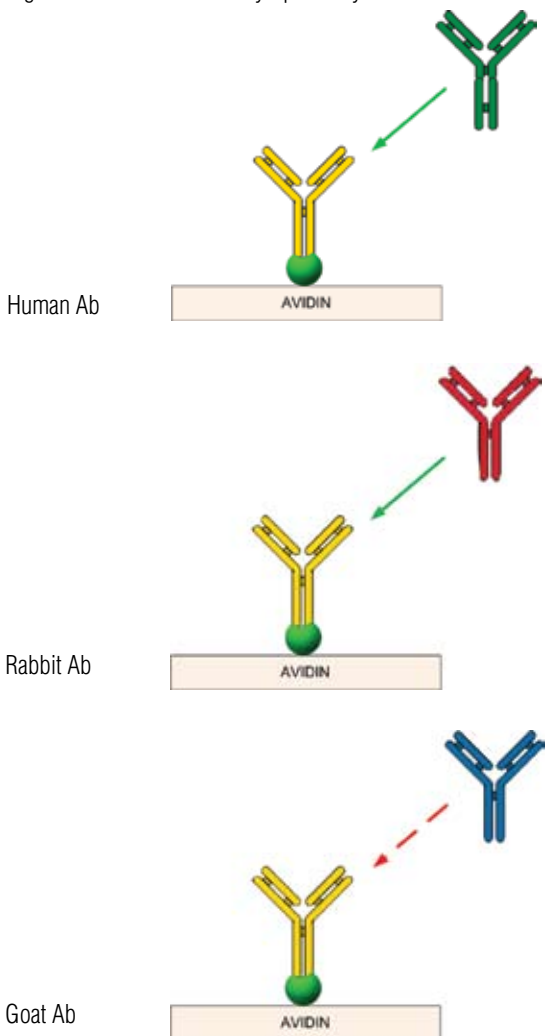


Figure 1: Real-Time Antibody Specificity



Introduction

Non-specific binding of the initial capture antibody can cause false positive results. Therefore, when developing a sandwich ELISA, testing the specificity (non-cross reactivity) of the initial capture antibody to prevent false positives is important. Testing antibody specificity is even more important when using a generic capture antibody or when developing an assay that employs multiple antibody species.

The assay shown here screens antibodies from three non-related species and then probes with the targeted species to confirm the activity of the antibody being studied.

Method

The following were sequentially immobilized onto the surface of an avidin dotLab® Sensor:

- 10 µg/mL biotinylated Goat anti-Human (bt Goat anti-Hu) antibody
- 10 µg/mL each of Rabbit, Goat, Mouse, then Human antibody

Blocking steps were used before each antibody injection at 5 mg/mL of BSA.

Results

- The real-time data trace showed significant cross reactivity of the bt. Goat anti-Hu with the Rabbit antibody.
- Goat and Mouse antibodies did not show obvious binding signal, and was negated for cross reactivity.
- The final load of Human antibody showed considerable binding, proving that bt Goat anti-Hu binds to its target.

In the overlaid trace,

- The binding curve of the specific species is clearly different from the binding curve of the cross-reactive species.
- The linear and then sloping curve of the cross-reactive (Rabbit) antibody suggests slow and non-specific binding.
- The curve of the target (Human) antibody has a quick initial binding, followed by equilibrium (flattened signal).

Conclusion

The dotLab® System screened four antibodies in a single assay, using a single sensor. The cross-reactive antibody (Rabbit) was quickly identified thereby enabling assay remedy e.g., adjusting the assay format or purifying the capture antibody to eliminate the cross reactivity.