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ELISA FAQs

1. What are the stabilities of the HRP conjugates included in PeproTech's ELISA Development Kits?

   The avidin-HRP included in the ABTS kits is stable for up to 1 month at 2-8°C, and up to 2 years at -20°C.

   The streptavidin-HRP included in the TMB kit is stable for at least 6 months at 2-8°C.

2. How can I find cross-reactivity information for a kit?

   PeproTech performs lot-specific, in-house cross-reactivity testing on its ELISA Development Kits. The results that have been collected from this testing are located on the kit's data sheet.

3. Is there any step of the ELISA protocol that can be left over the weekend?

   The plate may be coated with the capture antibody on Friday, left at 4°C over the weekend, and resumed on Monday. Please note: changing incubation times may cause results to vary between plates.

4. Are PeproTech's EDKs suitable to use with all sample types?

   Although PeproTech has not tested all of its kits in every matrix available, they should be suitable for use in, but not limited to: serum, plasma, cell culture supernatant, urine and saliva.

5. Is a stop solution necessary to stop the reaction?

   A stop solution is not needed when using avidin-HRP + ABTS. In general, reliable standard curves are obtained when either O.D. readings do not exceed 0.2 units for the zero standard concentrations, or 1.2 units for the highest standard concentration. If a stop solution is desired, 1% sodium dodecyl sulfate (SDS) may be used to end the reaction. Stop solutions are not used in PeproTech's laboratory with ABTS kits.

   A stop solution (1M HCI Stop Solution) is recommended with all of PeproTech's TMB kits.

6. In addition to the 620nm correction wavelength recommended for the TMB EDKs, can other wavelengths be used?

   A correction wavelength of 540, 570, 620, or 650nm can be used with the TMB EDKs.

7. Can I use TMB with PeproTech's ABTS EDKs?

   PeproTech's ABTS EDKs are optimized using ABTS and are, therefore, best used in conjunction with this substrate. The kit can still be used in combination with TMB, but only after some adjustments have been made:

   - The avidin-HRP provided in the kit cannot be used with TMB; streptavidin must be purchased separately.
   - Dilutions of streptavidin will need to be optimized.
   - A stop solution is generally needed when using streptavidin + TMB. Refer to manufacturer's data sheet.
   - The TMB reaction time, prior to the addition of stop solution, will need to be optimized.
   - The plate is to be read at 450nm with a correction wavelength at 620nm when using recommended plates.

8. Why is D-mannitol added to the EDK components?

   D-mannitol is added to the EDK components in order to aid in protein/antibody visualization. It does not alter ELISA results.

9. Can I use the curve on the EDK data sheet as my standard curve?

   A separate standard curve must be run on each ELISA plate. In other words, the curve from one plate cannot be used for a different plate. The curve that PeproTech provides on the EDK data sheet is for demonstration purposes only, as achieved in PeproTech's laboratory.

10. How does PeproTech generate their standard curve?

    When an ELISA is run in PeproTech's lab, a Molecular Devices® plate reader and the SOFTmax®PRO software are used. This program uses the values that are received and generates a 4-parameter curve. Here is the equation that the program uses:

    4-P Fit:

$$y = \frac{A - D}{1 + \left(\frac{x}{C}\right)^{B}} + D$$

x = concentration (pg/ml)
y = O.D. (405nm – 650nm)
A, B, C, & D correspond to the 4 parameters.*
*For more detailed information regarding the parameters, please contact the Quality Assurance Department.