R&D SYSTEMS

Spike and Recovery
Immunoassay Sample Validation Protocol

Experimental protocol only-not guaranteed

Buffer components, sample matrix, complement, heterophilic antibodies, and rheumatoid factor can impact accuracy of ELISA results. R&D Systems' ELISAs have been designed to minimize the impact of these factors on values reported from validated sample types. Before using R&D Systems' ELISAs to analyze any unvalidated sample type, spike/recovery and linearity experiments should be performed on these samples to determine whether sample values reported from unvalidated sample types are accurate. In spike/recovery assays, a known amount of ELISA standard is "spiked" into a sample. The resulting concentration, or "recovery" of the spiked material, indicates whether a component in the sample interferes in the ELISA. Spiked or unspiked samples are also serially-diluted to test for linearity; if a sample does not exhibit linear dilution, this indicates that a sample component is interfering with accurate detection of a specific analyte at a given dilution. It is important also to find the linear dilution range for an unvalidated sample type as this will allow comparison of sample values generated from samples run at different dilutions. Performing spike/recovery and linearity testing on unvalidated samples helps to show whether the values reported from these samples are accurate.

If an unvalidated sample type is to be tested using Quantikine or DuoSet assays, the following protocol can be used as a guide for testing spike/recovery and linearity.

I. Items required for Spike/Recovery Testing

- ELISA assay
- 10X Spiking Stock Solution
- 2.0 mL of a well-mixed sample with a value within the standard curve range (If the neat sample does not produce a value within the standard curve range, dilute it until the value reads within the standard curve range. Use this dilution for the spike/recovery and linearity assays.)

II. Spiking Stock Solution Preparation

Prepare a concentrated spiking stock solution by reconstituting the kit standard to 10X the recommended high standard concentration (use the diluent indicated in the ELISA protocol for reconstitution). For example, if the ELISA protocol states to use 5 mL Calibrator Diluent for preparation of the standard stock solution, reconstitute in 0.5 mL to generate a 10X spiking stock. Recombinant protein other than an ELISA standard can be used to generate a spiking stock solution, but using the ELISA standard as a spiking stock will eliminate the need to first mass assign recombinant protein to be used as a spiking stock.

III. Standard Curve Preparation

A standard curve can be prepared by diluting the 10X spiking stock 1:10 in the recommended Standard Curve Diluent.*

IV. Sample and Control Spike Preparation

1. Label 3 tubes, neat, spiked, and control.
2. From a well-mixed sample prepare two aliquots:
   a. Pipette 1.0 mL into a tube labeled "neat". This is the "neat" sample.
   b. Pipette 1.0 mL into a tube labeled "spiked". This will be used to generate the spiked sample.
3. Pipette 1.0 mL Standard Curve Diluent* into tube labeled "control."
4. Prepare the Control Spike and Sample Spike by pipetting an identical volume of 10X Spiking Stock Solution into an identical volume of each (i.e. add 20 µL 10X spiking stock into 980 uL Sample and 980 µL Control). The amount spiked should fall in the middle of the standard curve range indicated in the protocol. The Control Spike and Sample Spike must be prepared simultaneously using the same pipette, but take care to change pipette tips between spikes. The "Control Spike" will be used for comparison to the "Sample Spike." Be sure to have sufficient final volume for running duplicate samples of each.
5. Vortex spiked samples briefly.

*Refer to specific protocol for proper standard diluent. Standard diluent should mimic the sample matrix as closely as possible.

V. Preparing Sample/Control Spike Serial Dilutions (Testing Sample Linearity)
To test samples for linearity, make serial dilutions of the Sample Spike and Control Spike. If the neat sample has a value greater than 60% of the high standard, test the sample for natural linearity using the same dilution series described below. **Vortex briefly between each dilution.**

1. 1:2 dilution
   Add 0.5 mL of Sample Spike, Control Spike, or neat sample to 0.5 mL Standard Curve Diluent.

2. 1:4 dilution
   Add 0.5 mL of 1:2 dilution to 0.5 mL Standard Curve Diluent.

3. 1:8 dilution
   Add 0.5 mL of 1:4 dilution to 0.5 mL Standard Curve Diluent.

These dilutions will be read off the standard curve to determine if dilutions of unvalidated samples are parallel to the standard curve and if the values of the sample dilutions are accurate.

The plate could be set up as follows:

	1	2	3	4	5	6
A	Std 1		Spiked sample		Control spike	
B	Std 2		1:2 Spiked sample		1:2 Control spike	
C	Std 3		1:4 Spiked sample		1:4 Control spike	
D	Std 4		1:8 Spiked sample		1:8 Control spike	
E	Std 5		Neat sample			
F	Std 6		1:2 Unspiked sample			
G	Std 7		1:4 Unspiked sample			
H	blank		1:8 Unspiked sample			

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IV. Calculations

1. Spike/Recovery

$$\% \text{ Recovery} = \frac{\text{Observed - Neat}}{\text{Expected}} \times 100$$

Observed = Spiked sample value
Neat = Unspiked sample value
Expected = Amount spiked into sample

Note: The neat sample may read 0 pg/mL.
Recovery should be in the range of 80-120%.
Control Spike should have a recovery value within 80-120%. If not, this indicates there was a problem in preparing the Control Spike.

2. Linearity

Use the spiked sample value as the Expected Value if testing linearity of the spiked sample.
Use the neat sample value as the Expected Value if testing linearity of the unspiked sample.

$$\% \text{ Recovery (1:2)} = \frac{\text{Observed value (pg/mL) of 1:2 dilution}}{\text{Expected value (pg/mL) divided by 2}} \times 100$$

$$\% \text{ Recovery (1:4)} = \frac{\text{Observed value (pg/mL) of 1:4 dilution}}{\text{Expected value (pg/mL) divided by 4}} \times 100$$

$$\% \text{ Recovery (1:8)} = \frac{\text{Observed value (pg/mL) of 1:8 dilution}}{\text{Expected value (pg/mL) divided by 8}} \times 100$$

Note: Recovery of spiked/neat samples should be in the range of 80-120%.
Diluting the Control Spike is a good control for serial dilutions. Recovery for the Control Spike should be in the range of 80-120%. If not, this indicates there was a problem in preparing the Control Spike dilutions.

Conclusion:

If the spike recovery and linear dilution studies are in the proper range, confidence of using the ELISA kit for the unvalidated sample is increased. In our Assay Development labs, many additional assays over several kit lots are performed for full validation of a specific sample type and several samples are assayed in these formats. Additionally, interfering substances are tested as are potential cross-reactants.