Online Training Curriculum

Confirmation Methods for Food contaminants

Principles of ELISA

Troubleshooting

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Overview

Introduction

Several issues my be faced while performing ELISA test and will be developed in the following document. Apart from non-specific binding and background noise, high or low signal readouts, incomplete color development, poor reproducibility, inconsistency in the control readouts, and non-linear calibration curves are some examples. It is helpful then to:

- Write down the entire personalized protocol and deviations prior to conducting the assay.
- Label and arrange the samples, the buffers, the well plates etc., and have a clear mapping of the experiment in advance.
- Develop pipetting skills and a good concentration on the assay.
- Do a careful checking of the equipment such as incubators, fridges, and readout machines before planning an assay. Pipettes need to be calibrated on a regular basis as well [1].

Troubleshooting of ELISA assay is well documented. Several kit producers propose solution on their website (check references).

Weak or no signal in ELISA

No signal in your ELISA assay may be the results of several potential issues listed below. Weak signal in ELISA can lead to inaccurate results and potential false negative results. If samples are weak but standards samples are close to the quality control curve given with the kit, sample preparation may be the root of the problem.

Possible Cause	Solution
Reagents not at room temperature at start of assay	It is recommended that all reagents be at room temperature before starting the assay. Allow reagents to sit on bench for 15-20 minutes to reach room temperature.

Storage of components	Double check storage conditions on kit label. Most kits need to be stored at 2-8°C.
Expired reagents	Confirm expiration dates on all reagents. Do not use reagents that are past the expiration date.
Reagents added/prepared incorrectly	Check protocol, ensure reagents were added in the proper order and prepared to correct dilution.
Incorrect dilutions prepared	Check pipetting technique and double check calculations.
Not enough detector antibody used	Manufactured kits have optimized protocols. Make sure to follow recommended antibody dilutions.
Wells scratched	Use caution when dispensing and aspirating into and out of wells. Automated plate washers may need to be calibrated so tips do not touch bottom of wells.
Plate read at incorrect wavelength	Manufactured kits have optimized protocols. Make sure to use recommended wavelength. Ensure plate reader is set accurately for type of substrate being used.
Substrate incubation carried out in the light	Substrate incubation should be carried out in the dark. Ensure substrate is not exposed to light - store in a dark place. Limit exposure to light while running assay.

In some rare cases, defective kits may reach the market. In that case, a weak signal could be the sign an incomplete binding of antibody to the microplate. If any of the solution proposed above works, it could be a good idea to reach the manufacturer of the kit to verify if the lot product does not present quality issues.

Poor standard curve in ELISA

A poor standard curve will prove unusable results if not prepared correctly. Reasons may include reagents are poorly mixed, the standard has degraded or pipetting errors

Possible Cause	Solution
Incorrect standard curve dilutions prepared	Check pipetting technique and double-check calculations.
Storage of components	Double check storage conditions on kit label. Most kits need to be stored at 2-8°C.
Expired reagents	Confirm expiration dates on all reagents. Do not use reagents that are past the expiration date.
Reagents added/prepared incorrectly	Check protocol, ensure reagents were added in the proper order and prepared to correct dilution.

Too much signal in ELISA

High Signal can occur for numbers reasons including insufficient plate washing, not stopping the reaction and adding too much detection reagent. If you have a high signal this may lead to false positive results and incorrect data.

Possible Cause	Solution

Insufficient washing	Use appropriate washing procedure. At the end of each washing step, invert plate on absorbent tissue and allow to completely drain, using tapping with force to remove any residual fluid.
Plate sealers not used or reused	During incubations, you may cover assay plates with plate sealers. Use a fresh sealer each time the plate is opened.
Incorrect dilutions prepared	Check pipetting technique and double-check calculations.
Longer incubation times than recommended	Manufactured kits have optimized protocols. Make sure to follow recommended incubation times.
Reaction not stopped	Colour will keep developing if the substrate reaction is not stopped.

High background in ELISA

High background may result from inadequate washing steps, cross reactivity of samples or contamination. High background may result in false positive/negative data and affect your results.

Possible Cause	**Solution**
Insufficient washing	Use appropriate washing procedure. Increasing duration of soak steps may also help. At the end of each washing step, invert plate on absorbent tissue and remove any residual fluid.
Substrate exposed to light prior to use	Ensure substrate is not exposed to light and stored in a dark place. Limit exposure to light while running assay.
Longer incubation times than recommended	Manufactured kits have optimized protocols. Make sure to follow recommended incubation times.

Incorrect standard curve dilutions prepared	Check pipetting technique and double-check calculations.
Matrix interference	Check the matrix ingredients for cross reacting components

Poor replicate data or inconsistent results assay-to-assay in ELISA

A high relative standard deviation is often linked to issues with sample preparation, but several ELISA laboratory practices may be in cause.

Possible Cause	**Solution**
Insufficient washing	Use appropriate washing procedure. Increasing duration of soak steps may also help. At the end of each washing step, invert plate on absorbent tissue and remove any residual fluid.
Plate sealers not used or reused	Use a fresh sealer each time the plate is opened. This will prevent wells from contaminating each other.
Inconsistent pipetting	Ensure pipettes are working correctly and are calibrated. Ensure pipette tips are pushed on far enough to create a good seal. Take particular care when diluting down the plate and watch to make sure the pipette tips are all picking up and releasing the correct amount of liquid.
Non-homogenous	Thoroughly mix samples before pipetting
Samples may have high particular matter	Remove the particulate matter by centrifugation

Insufficient plate agitation	The plate should be agitated during all incubation steps using an ELISA plate shaker at a speed where solutions in wells are within constant motion without splashing
Inconsistent experimental conditions	Be aware of fluctuations in temperature or humidity due to environmental conditions. Make sure to follow recommended incubation temperatures if necessary.
Incorrect dilutions	Check pipetting technique and double-check calculations.

Edge effects in ELISA

The edge effect results in irregular results for the wells located at the microplate borders.

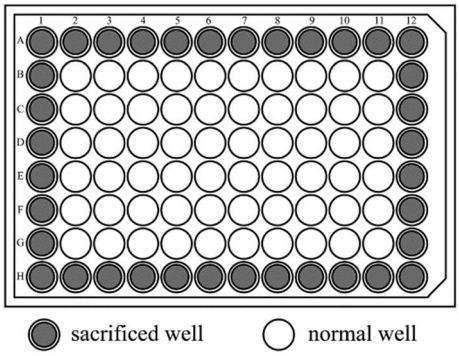

Figure 1: Wells impacted by edge effects [2]

Possible Cause	Solution
Uneven temperature	Seal the plate completely with a plate sealer during incubations. If high temperature of incubation is indicated make sure plate is in the center of incubator.

Evaporation	Seal the plate completely with a plate sealer during incubations.
Stacked plates	Avoid stacking plates during incubation.

References

1. Hosseini et al. (2018). Advantages, disadvantages and modifications of conventional ELISA, in Enzyme-linked Immunosorbent Assay (ELISA), Springer, Pages 67-115.

2. Wang & Cheng (2015). An enzyme-linked immunosorbent assay with a new way to control the edge effect and its application for bevacizumab pharmacokinetic studies in beagle dogs by fitting with a new pharmacokinetic model. Analytical Methods, 7: 8936-8941.

The following references are not explicit in the text but were used for research purposes.

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