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CUSABIO®

ELISA

How to Solve the Common Problems in ELISA?

ELISA Troubleshooting Tips

CUSABIO, as a good partner in your research, offers these troubleshooting tips to solve your ELISA problems, including common causes of high background or non-specific color development, no color development, weak signal, poor reproducibility and more.

High Background or Non-specific Color Development

Problem description: After the reaction is terminated, the whole plate appears uniform yellow or light yellow; or the standard curve is linear but the background is too high.	
Possible cause	Solution
Wrong reagents were added.	Make sure the reagents used in experiment come from the same kit.
Wells were insufficiently washed.	Wash wells as recommended in the protocol.
Longer incubation time than recommended.	Strictly follow the instructions.
Wells were contaminated.	Avoid cross-well contamination by using the plate sealer appropriately. Use multichannel pipettes without touching the reagents on the plate.
Too much antibody/ HRP-avidin used.	Check the dilution factor of antibody/HRP-avidin or dilute further.
TMB Substrate Solution was contaminated.	TMB Substrate Solution should be colorless or light blue and stored in the dark prior to addition to wells.
Longer color development time than recommended.	Strictly follow the instructions to control the color development time.
Wrong settings on the plate reader.	Check the wavelength and read the plate again. Measure absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm

No Color Development

Problem Description: After the color development step, all the wells of the plate have no color. Positive control does not develop color.	
Possible cause	Solution
Reagents were used in the wrong order or an assay step, reagent or antibody was omitted.	Check the package insert for the assay protocol and repeat the assay.
During the process of plate washing and sample addition, HRP-avidin or HRP-conjugate lost activity.	HRP-avidin or HRP-conjugate doesn't contain enzyme inhibitor, such as NaN3 etc. Make sure the container for the wash buffer is clean.

Weak Signal

Problem Description: The color of all plate wells is light, including the standard curve and the sample.	
Possible cause	Solution
The kit has expired or was not stored properly.	Confirm that the kit is within the validity period and store it in the storage conditions recommended in the protocol to avoid contamination.
Reagents and samples were not balanced before use.	Bring all reagents to room temperature (18-25°C) before use for 30min.

The pipette was operating incorrectly.	Strictly follow the instructions.
Incubation time too short.	Strictly follow the instructions.
The color reaction time was insufficient.	The color development time is generally 15-30min, 20min is better.
The order of adding the substrate was reversed.	Strictly follow the instructions.
Too many washing times, and the dilution factor of concentrated wash buffer was wrong.	Strictly follow the instructions to dilute the concentrated wash buffer, and accurately record the washing times and dosage.
Unqualified distilled water.	The prepared distilled water must be tested for neutral pH.
Contamination of solutions.	Make fresh solutions.
Problem Description: The standard curve is normal, but the sample develops light color.	
Possible cause	Solution
The sample was stored with NaN3, which inhibits the enzyme reaction.	Samples cannot be stored with NaN3.
The sample may not contain strong positive analyte, so the test result may be normal.	Repeat the assay if any doubts.
Problem Description: The reading of the plate reader is low.	
Possible cause	Solution
Wrong settings on the plate reader.	Check plate reader for wavelength, filters, gain etc. and read plate again.

Poor Standard Curve and Reproducibility

Problem description: Poor reproducibility	
Possible cause	Solution
Standard solution has not been prepared correctly.	Strictly follow the instructions, and confirm dilutions are made correctly.
Inappropriate storage.	Ensure all samples are stored according to recommendations. Do not leave the reconstituted components at room temperature for too long.
Dilute each working component too early.	Please prepare each working component 10 minutes before use and add it to the micro-well immediately.
The sample was not mixed well after addition.	When adding multiple reagent components at the same time, mix thoroughly on the mixer after adding the sample.
The plate reader has poor reproducibility.	Calibrate the plate reader.
Inconsistency in incubation time, washing and color development conditions.	Repeat the assay, and keep the reaction conditions as consistent as possible with the last time.
Incorrect washing.	Strictly follow the instructions.
Incubation temperature not constant.	Ensure that the temperature is constant and avoid local temperatures that are too high or too low.

Too much remains on the wall of the plate well.	Add liquid along the bottom of the well wall without touching the bottom of the well.
Negative and positive around the threshold.	Run triplicated wells for the same sample, and take two (including two or more) same results as the standard.
Cross-well contamination.	Take care when using same pipette tips for reagent additions. Ensure that pipette tips do not touch the reagents on the plate.
Problem Description: Random drifted wells appear and test values are abnormal.	
Possible cause	Solution
Cross contamination caused by manual plate washing.	Take care when using same pipette tips for reagent additions. Ensure that pipette tips do not touch the reagents on the plate.
Inconsistent washing or washer system malfunctioning.	Make sure the washer system works well. Have the system repaired if there is any problem.
Insufficient centrifugation of the sample caused coagulation in the well or interference with sediment or residual cellular components.	Serum and plasma should be fully centrifuged at 3000rpm for 6min or more.
The sample was stored for a long time and was contaminated.	The samples should be kept fresh or kept at low temperature to prevent contamination.
Wash buffer prepared incorrectly.	Follow the instructions.

Why Choose ELISA Kits from CUSABIO?

Ready-to-use

No special handling required.
Simple operation.

Cited in More Than 8000 References

CUSABIO ELISA kits are cited in more and more publications in well-known magazines such as *Nature, Cell, Nature Medicine, Cell Metabolism, Nature Immunology, Gastroenterology* and so on.

Multiple Species

CUSABIO ELISA kits cover more than 20 species, including human, mouse, rat, bovine, rabbit, pig, sheep, goat, monkey, etc.

Multiple Biological Samples

CUSABIO ELISA kits are validated in multiple biological samples, including serum, plasma, body fluids, cell culture supernatant, saliva, etc.

High Performance

High-standard quality control is performed from raw materials to delivery to ensure product quality.

Top 50 Hot Selling ELISA Kits

Target	Code	Species	Types of Sample	Sensitivity	Detect Range	Number of Publications
TNF	CSB-E11987r	Rat	serum, plasma, cell culture supernates, tissue homogenates	1.56 pg/mL	6.25 pg/mL-400 pg/mL	254
TNF	CSB-E04741m	Mouse	serum, plasma, cell culture supernates, tissue homogenates	3.9 pg/mL	15.6 pg/mL-1000 pg/mL	188
IL6	CSB-E04640r	Rat	serum, plasma, tissue homogenates	0.078 pg/mL	0.312 pg/mL-20 pg/mL	182
IL6	CSB-E04639m	Mouse	serum, plasma, cell culture supernates	0.39 pg/mL	1.56 pg/mL-100 pg/mL	145
IL1B	CSB-E08055r	Rat	serum, plasma, cell culture supernates, tissue homogenates	15.6 pg/mL	62.5 pg/mL-4000 pg/mL	144
IL6	CSB-E04638h	Human	serum, plasma, cell culture supernates, tissue homogenates, urine	2.453 pg/mL	7.8 pg/mL-500 pg/mL	125
IL1B	CSB-E08054m	Mouse	serum, plasma, cell culture supernates, tissue homogenates	7.8 pg/mL	31.25 pg/mL-2000 pg/mL	125
TNF	CSB-E04740h	Human	serum, plasma, cell culture supernates, tissue homogenates, cell lysates	1.95 pg/mL	7.8 pg/mL-500 pg/mL	87
CASP3	CSB-E08857r	Rat	serum, plasma, tissue homogenates	0.078 ng/mL	0.312 ng/mL-20 ng/mL	87
INS	CSB-E05071m	Mouse	serum, plasma, cell culture supernates, tissue homogenates, cell lysates	3.9 nIU/mL	15.6 nIU/mL-1000 nIU/mL	80
IL10	CSB-E04595r	Rat	serum, plasma, cell culture supernates, tissue homogenates	0.78 pg/mL	3.12 pg/mL-200 pg/mL	76
T	CSB-E05100r	Rat	serum, plasma, cell culture supernates, tissue homogenates	0.06 ng/mL	0.13 ng/mL-25.6 ng/mL	75
FSH	CSB-E06869r	Rat	serum, plasma, tissue homogenates	0.07 mIU/mL	0.3 mIU/mL-10 mIU/mL	75
LH	CSB-E12654r	Rat	serum, plasma	0.15 mLU/mL	0.3 mLU/mL-60 mLU/mL	71
IgM	CSB-E12045Fh	Fish	serum, plasma, tissue homogenates	1.25 μg/mL	1.25 μg/mL-50 μg/mL	63
INS	CSB-E05070r	Rat	serum, plasma, cell culture supernates	3.9 nIU/mL	15.6 nIU/mL-1000 nIU/mL	62
LPS	CSB-E13066m	Mouse	serum, plasma, tissue homogenates	0.039 ng/mL	0.156 ng/mL-10 ng/mL	51
IL1B	CSB-E08053h	Human	serum, cell culture supernates, urine, cerebrospinal fluid (CSF)	31.25 pg/mL	125 pg/mL-8000 pg/mL	51
VEGFA	CSB-E11718h	Human	serum, plasma, cell culture supernates, tissue homogenates, cell lysates, urine	25.297 pg/mL	31.25 pg/mL-2000 pg/mL	49
IL10	CSB-E04594m	Mouse	serum, plasma, cell culture supernates, tissue homogenates	0.78 pg/mL	3.12 pg/mL-200 pg/mL	48
hs-CRP	CSB-E08617h	Human	serum, plasma, cell culture supernates, tissue homogenates	0.156 ng/mL	0.625 ng/mL-40 ng/mL	45
TSH	CSB-E05115r	Rat	serum, plasma, tissue homogenates, cell lysates	0.3 μIU/mL	0.6 μIU/mL-24 μIU/mL	39
IFNG	CSB-E04578m	Mouse	serum, plasma, tissue homogenates, cell culture supernates	3.9 pg/mL	15.6 pg/mL-1000 pg/mL	39
E2	CSB-E05110r	Rat	serum, plasma	40 pg/ml	40 pg/ml-1000 pg/ml	39
TGFB1	CSB-E04725h	Human	serum, plasma, cell culture supernates	0.747 ng/mL	0.78 ng/mL-50 ng/mL	38

Target	Code	Species	Types of Sample	Sensitivity	Detect Range	Number of Publications
ANG- II	CSB-E04494r	Rat	serum, plasma, cell culture supernates, tissue homogenates, cell lysates	1.17 pg/mL	4.7 pg/mL-300 pg/mL	37
CORT	CSB-E07014r	Rat	serum, plasma, tissue homogenates	0.1 ng/mL	0.2 ng/mL-40 ng/mL	35
BCL2	CSB-E08854r	Rat	serum, plasma, tissue homogenates	0.078 ng/mL	0.312 ng/mL-20 ng/mL	34
VEGFA	CSB-E04757r	Rat	serum, plasma, tissue homogenates	0.97 pg/mL	3.9 pg/mL-250 pg/mL	33
TGFB1	CSB-E04727r	Rat	serum, plasma, cell culture supernates, tissue homogenates	1.56 pg/mL	6.25 pg/mL-400 pg/mL	33
LPS	CSB-E09945h	Human	serum, plasma, tissue homogenates	1.56 pg/mL	6.25 pg/mL-400 pg/mL	33
CK-MB	CSB-E14403r	Rat	serum, urine, tissue homogenates, plasma	0.078 ng/mL	0.312 ng/mL-20 ng/mL	33
CAT	CSB-E15928Fh	Fish	serum, plasma	62.5 mU/mL	125 mU/mL-2000 mU/mL	33
8-OHdG	CSB-E10140h	Human	serum, urine	3.12 ng/mL	3.12 ng/mL-800 ng/mL	33
SOD	CSB-E15929Fh	Fish	serum, plasma, tissue homogenates	0.625 ng/mL	1.25 ng/mL-20 ng/mL	31
IL10	CSB-E04593h	Human	serum, urine, cell culture supernates, ascitic fluid, cerebrospinal fluid (CSF), saliva	3.12 pg/mL	12.5 pg/mL-800 pg/mL	31
MMP9	CSB-E08008r	Rat	serum, plasma, tissue homogenates	0.195 ng/ml	0.78 ng/ml - 50 ng/ml	30
IFNG	CSB-E08550Ch	Chicken	serum, plasma, tissue homogenates	3.125 pg/mL	3.125 pg/mL-800 pg/mL	30

Target	Code	Species	Types of Sample	Sensitivity	Detect Range	Number of Publications
DA	CSB-E08660r	Rat	serum, plasma, tissue homogenates	0.039 ng/mL	0.156 ng/mL-10 ng/mL	30
CCL2	CSB-E07430m	Mouse	serum, plasma, tissue homogenates	19.5 pg/mL	78 pg/mL-5000 pg/mL	30
ADP	CSB-E07270h	Human	serum, plasma, cell culture supernates, tissue homogenates, urine	1.102 ng/mL	1.562 ng/mL-100 ng/mL	30
IL6	CSB-E06903Rb	Rabbit	serum, plasma, tissue homogenates	3.9 pg/mL	15.6 pg/mL-1000 pg/mL	29
Aβ1-42	CSB-E10684h	Human	serum, plasma, tissue homogenates, cerebrospinal fluid (CSF)	0.078 ng/mL	0.312 ng/mL-20 ng/mL	29
IL35	CSB-E13126h	Human	serum, cell culture supernates, urine, tissue homogenates	15.6 pg/mL	62.5 pg/mL-4000 pg/mL	28
COR	CSB-E08487f	Fish	serum, plasma, tissue homogenates	0.0023 ng/mL	0.0023 ng/mL-10 ng/mL	28
CD81	CSB-EL004960HU	Human	serum, plasma, tissue homogenates, cell lysates	0.039 ng/mL	0.156 ng/mL-10 ng/mL	28
PG-E2	CSB-E07967r	Rat	serum, plasma, tissue homogenates, cell lysates	0.25 pg/mL	0.5 pg/mL-100 pg/mL	27
LEP	CSB-E07433r	Rat	serum, plasma, tissue homogenates	0.068 ng/ml	0.068 ng/ml - 50 ng/ml	27
IL4	CSB-E04634m	Mouse	serum, plasma, tissue homogenates, cell culture supernates	0.39 pg/mL	1.56 pg/mL-100 pg/mL	27
IL18	CSB-E04609m	Mouse	serum, plasma, cell culture supernates, tissue homogenates	0.39 pg/mL	1.56 pg/mL-100 pg/mL	27