Quality & Safety

ELISA trouble shooting

Name:

Address:

Phone: Fax: Email:

What could be wrong if a test «did not work»?

If no color reaction or too low OD values are obtained?

Procedure was not followed correctly (manipulation error, e.g. step omitted)
Wrong buffer: pH, old buffer, contaminated buffer
Error in concentration of reagents
- due to wrong calculation or pipetting
- due to droplets of condensation stuck to the lid or wall of the vial (needs centrifugation)

Wrong storage of reagents
No positive control, no infected sample (wrong tissue or wrong season for detection)
Wells have dried between steps
Wrong microtiter plate
ELISA reader malfunctioning (e.g. wrong or damaged filter)
Quality of substrate
Faulty reagents (activity of antibodies, contamination)

If irregular color or non-specific reactions are obtained?

Incomplete washing and/or spillage between wells (e.g. malfunction of washer)
Error in concentration of reagents
- due to wrong calculation or pipetting
- due to droplets of condensation stuck to the lid or wall of the vial (needs centrifugation)

Mistake in application of reagents (e.g. conjugate instead of coating)
Edge effect due to improper incubation conditions
Wells have dried between steps
Old substrate, or substrate of poor quality
Faulty reagents (e.g. contamination of coating IgG with conjugate)
Faulty microtiter plate

We recommend to go through the following checklist in order to help in pinpointing possible causes of not obtaining satisfactory results.

Checklist:	yes	no
• Washing done correctly, automatic washer verified	○	○
• Recommended procedure followed	○	○
• Correct buffers used, fresh buffers	○	○
• Correct concentration of reagents used	○	○
• Fresh substrate used	○	○
• Functioning of ELISA reader verified	○	○
• OD values from ELISA reader correlate with visual reading	○	○
• Buffers were stored at +4° C but used at room temperature	○	○
• Reagents have been stored at +4° C	○	○
• Incubation temperatures used according to recommended procedure	○	○
• Microtiter plates covered during incubations	○	○
• Samples were loaded according to pre-determined pattern	○	○
• Reliable positive and negative controls used on each plate	○	○
• Replication of test samples (e.g. duplicate wells) gave similar readings	○	○

If your answers in the check list above are yes and you are sure that any "handling mistakes" can be ruled out, you may suspect the cause of the problem in your test with the reagents. Filling out the «complaint sheet» can help us finding out the problem of reagents.

Clear Form

Send to admin@bioreba.ch

www.bioreba.com

BIOREBA AG — Your Partner in Agro-Diagnostics
Christoph Merian-Ring 7
CH-4153 Reinach BL1
Switzerland
phone +41 61 712 11 25
fax +41 61 712 11 17
admin@bioreba.ch
www.bioreba.com