VIROTECH Diagnostics GmbH ELISA System Diagnostic Troubleshooting Guide ELISA EN

Error Sources and Approaches

The following questionnaire shall help to identify the error source and provide the approach as quickly as possible. It is not assay specific but valid for all our ELISAs. If the concern cannot be solved following this questionnaire, please do not hesitate to contact us at phone: +49 6074 23698-0

We will be pleased to assist you!

Your VIROTECH Diagnostics Team

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Chapter 2 Results are too high

Observation:
The controls exceed the range mentioned in the QC certificate

Sources of Error	Approach
Contaminated TMB	Check colour of the TMB (TMB is to be colourless)
Exceeded incubation times	Check the incubation time of the automatic device (30'-30'-30')
Temperature too high	Check the temperature of the incubator Automatic Device: Check the temperature of the incubators (37°C)
Use of wrong reagents	Check the reagents
Use of wrong controls resp. standards	Consider the correct lot number of controls resp. standards
Use of the wrong reference filter	Check the used wavelength (450nm/620nm)
Use of the wrong wash programme	Check the setting of the automatic device: 2 washing steps a 4 x with 350-400µl wash buffer
Poor quality of the aqua dest./deionised	Check water installation. Repeat the test with industrial filled aqua dest./deionised
Contaminated washing buffer	Check the pH-value Check the expiration date of the prepared washing buffer: 4 weeks at room temperature

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Contaminated washing buffer container	Clean well the washing buffer container, Fill in new diluted washing buffer; Avoid permanent re-filling
Clogged manifold	Eliminate the clogging
Incorrect manifold position	Justify the manifold

Chapter 3 Results are too low

Observation:
Controls below the range mentioned in the quality control certificate

Sources of Error	Approach
Contaminated conjugate	• Already smallest amounts of serum may lead to the inactivation of the conjugate. • Prepare only the amount of conjugate needed for the test run. • Do **not return** conjugate of the already withdrawn quantity back to the vial
Incubation time too short	Check incubation time (30‘-30‘-30‘)
Incorrect temperature	Check the temperature of the incubator Automatic Device: Check the temperature of the incubators (37°C)
Used expired components	Check expiration date of used components
Use of the wrong reference filter	Check the used wavelength (450nm/620nm)

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Used reagents too cold while testing procedure	Make sure to bring up reagents to room temperature before starting test procedure
Poor quality of the aqua dest./deionised	Check water installation. Repeat the test with industrial filled aqua dest./deionised
Excessive washing	Manual Testing + Washer: Check the washing device and programm (washing steps and used volume) Manual Testing: Check the setting of the used pipettes Test with automatic device: Check the setting of the washing comb and programm: 2 washing steps a 4 x with 350-400µl wash buffer
Use of wrong controls resp. standards	Consider the correct lot number of controls resp. standards
Incorrect manifold position	Justify the manifold
Remaining cleaning solution in the system	Purging well all container and tubes

Chapter 4 Poor Double-Determination

Sources of Error	Approach
Insufficient mix of sera and buffer	All reagents must be shaken before use
Non-calibrated pipettes	Check the volume of the pipette, calibrate if necessary
Mistakes during the washing procedure	Check the washing steps Check channels of your washing device/clean washing comb
Carry-over during the pipetting operation	Perform an additional manual testing

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Chapter 5 All cavities are yellow coloured

Sources of Error	Approach
Coloured TMB (bluish)	TMB is sensitive to light and must be stored in dark. It must be colourless before used for testing
Contaminated reagents	Check reagents: colour and eventually a turbidity is present?
Wrong storage of the kits	Check the storage room and storage temperature (2-8°C)
Inefficient Washing	Check the following: - Setting of the washing device, - The washing comb of used automatic device, it's position and program In case of manual testing: - The pipettes Washing: 4 x with 350-400µl washing buffer - Remove residues by tapping the plate carefully on a cellulose pad

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Chapter 6 Missing colour reaction inside the wells

Sources of Error	Approach
Washing step after TMB incubation - WRONG! -	Check washing steps. After TMB incubation no further washing step must be proceeded but stopping with stop solution!
Contaminated conjugate	Check conjugate by mixing conjugate and TMB (same amounts each) – a blue-purple colour change shall take place. • Prepare only the amount of conjugate needed for the test run. • Do **not return** conjugate of the already withdrawn quantity back to the vial • Already smallest amounts of serum may lead to the inactivation of the conjugate.
Faulty storage of the kit	Check the storage room and the storage temperature (2-8°C)
Excessive Washing (too much washing buffer used)	Check: - The washing device, - The washing comb of used automatic device, - Pipettes in case of manual testing. Washing: 4 x with 350-400µl washing buffer

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