VIROTECH Diagnostics GmbH Immunoblot System Diagnostic

Troubleshooting Guide Immunoblots EN

Error Source and Approaches

The following questionnaire shall help to identify the error source and the approach as quickly as possible.

If the concern cannot be solved following this questionnaire please do not hesitate to contact us at +49 6074 23698-0

We will be pleased to assist you!

Your VIROTECH Diagnostics Team

Chapter 1 General Guidelines

- ⇒ Bring up reagents to room temperature prior to starting the test run
- ⇒ The cut-off controls are lot specific
- ⇒ All required test function controls must appear (serum control band and corresponding conjugate control band). Otherwise the test run is invalid and must be repeated
- ⇒ Store developed strips in dark, as the bands fade in the course of time
- Chapter 2 Some or all strips are blank or only partially developed
- Chapter 3 Strips with "spots" or very dark developed strips
- Chapter 4 Cut-off or positive control do not react on the strip



Chapter 2 Some or all strips are blank or only partially developed

Observation:

White strips, no bands, test function control band did not appear

Error Source	Approach
No serum or no control(s) have been pipetted or the amount used was insufficient (wrong dilution)	 Serum control bands did not appear or appeared with a too low intensity Correct Dilution: 15µl serum resp. 100µl control + 1,5 ml serum dilution buffer Please consider exceptions!
conjugate/substrate has not been pipetted or	 Serum control bands did not appear or appeared with a too low intensity Check conjugate dilution (1+100)
mistake in conjugate dilution	Automatic Devices: Take care that the tubes are located in the correct container and check for programming errors
Prepared conjugate dilution lost reactivity	Prepare only the needed amount of conjugate prior to each test run. (Stability of diluted conjugate: 6h at 2-8°C)
Use of wrong reagents	Check used reagents
Too short incubation times	Check incubation times Check setting of the automatic device used
Strips are not completely moistened (probably bent strips)	 Detach strips carefully out of the booklet, only at the strip numbering Take care that all strips are moistened from beginning of the test run! Strips must not dry out during the test run
Error in washing procedure	Check the single washing steps Check setting of the automatic device used
Poor quality of the Aqua dest./deionised	Check water installation



Contaminated washing buffer	Check pH-value
Exceeded expiry date of the diluted washing buffer	Consider stability
Too cold reagents used for test run	Bring up reagents to room temperature before starting the test run (18-25°C)
Automatic Device: polluted tubes/ tubes containing liquid residues	Rinse tubes with aqua dest. thoroughly, if necessary disinfect on a daily basis - most suitable at the end of the working day

Chapter 3 Strips with "spots" or very dark developed strips

Observation:

Purple coloured strips, "spots" or "clouds" on the strips

Error Source	Approach
Too cold reagents used for test run	Bring up reagents to room temperature prior to starting the test run
Exceeding of pre-given incubation times	Check incubation times
Room temperature over 25°C	Adjust the temperature (18-25°C)
Washing steps too short resp. less washing steps than pre-given in the IFU	Check washing steps
Contaminated washing buffer	Check pH-value
Exceeded expiry date of the diluted washing buffer	Consider stability
Missed out the aqua dest. steps	Check washing steps:
	 1x 1 min. aqua dest. prior to the substrate incubation
	stop test: 3x with aqua dest.



Additional washing step has been proceeded after substrate incubation	 Check washing steps: no wash step must be proceeded after the substrate incubation stop test: 3x with aqua dest.
Contaminated conjugate dilution	Prepare only the needed amount of conjugate prior to each test run.
Polluted incubation tray	Take care to always use clean trays
Finger prints on the nitrocellulose, while treating test strips without wearing gloves	Always wear gloves and best take the strips with a plastic tweezer at the strips numbering part
Automatic Device: polluted tubes/ tubes containing liquid residues	Rinse tubes with aqua dest. thoroughly, if necessary disinfect on a daily basis - most suitable at the end of the working day

Chapter 4 Cut-off or positive control do not react on the strip

Observation:

Cut-off resp. positive control develops too weak or does not appear at all

Error Source	Approach
Too cold reagents used for test run	Bring up reagents to room temperature (18-25°C) prior to starting test run
Too short incubation times	Check incubation times (check programme settings of the automatic device)
Incorrect lot numbers used	Use only the cut off control delivered inside the kit or fitting to the kit lot in question. The positive control is lot independent
Serum control bands did not appear or were developed too weak	 Check if: Check if controls have been pipetted Check if correct dilution has been used: 100µl control + 1,5 ml serum dilution buffer



Conjugate control bands did not appear or were developed too weak	 Check if conjugate has been pipetted Check if correct dilution has been used Automatic Devices:
	Take care that the tubes are located in the correct container and check for programming errors
Strips are not completely moistened (probably bent strips)	 Detach strips carefully out of the booklet Take care that all strips are moistened from beginning of the test run! Strips must not dry out during the test run
Automatic Device: polluted tubes/ tubes containing liquid residues	Rinse tubes with aqua dest .thoroughly, if necessary disinfect on a daily basis - most suitable at the end of the working day

