CANDOR
The ELISA Experts

TROUBLESHOOTING

Problem	Probable cause	Solution
Appearance of smear on the membrane.	The transfer buffer has not been washed out completely before starting incubation with the antibodies.	Optimize the washing step. Note that the membrane is fully moistened and free floating in the Wash Buffer. If necessary please increase shaking during washing.
There are black spots on the membrane.	Appearance of black spots on Western blotting membranes is a common problem. The reasons are multifactorial and can only be solved by optimizing all working steps. Choice of substrate is important.	Use the ReadyTector® solution as described and with the corresponding ReadyTector® Wash Buffer and ReadyTector® Chemiluminescent Substrate. Use enough solution for the washing and incubation steps to enable the membrane to float in the solution. In this way spots can be mostly reduced. The ReadyTector® Chemiluminescent Substrate has been optimized to reduce the number of spots in comparison to most other substrates. But separate spots cannot be excluded.
Appearance of background on the membrane.	Washing has not worked.	Always wash carefully with the special ReadyTector® Wash Buffer. Note that the membrane is fully moistened and free floating in the Wash Buffer. Shake the membrane during washing so that the membrane can freely float in the solution. Wash 4 times after incubation with the antibody containing ReadyTector® solution.
	Blocking has not worked due to incorrect washing step before incubation with the antibodies.	Optimize washing step. See above.
	Blocking has not worked.	Use an adequate volume of ReadyTector® solution for incubation of the membrane. Ensure that the membrane is fully moistened and free floating in the solution. 20 mL for a membrane with the dimensions 8 × 10cm is sufficient. Less solution can cause problems.
	The membrane has been too long exposed to X-ray film or imager after detection with the substrate.	Reduce exposition time. In most cases the background will be lower with ReadyTector® compared to the standard protocol. The ReadyTector® Chemiluminescent Substrate is a sensitive and fast substrate. Thus exposition times can be reduced. Instrument settings have to be adjusted.

CANDOR
The ELISA Experts

TROUBLESHOOTING

Problem	Probable cause	Solution
No visible bands. The membrane doesn't show background.	The primary antibody binds only with low affinity to the target. Thus it is not able to bind the target protein in ReadyTector®. The blocking has worked and so no background is visible on the membrane.	The best solution would be to change the primary antibody against a better antibody with higher affinity. If this is not possible, extend the incubation time up to 2 hours or more. If the result is still bad, switch to the standard procedure and forget the fast one-step immunodetection with ReadyTector®. Some antibodies are not useful for fast immunodetection just due to low affinity of their binding.
	The protein is not on the membrane.	Check your protein solution, the gel run and the blotting procedure. Check if your protein has been transferred to the membrane.
The desired bands are ok but there are additional bands visible.	The antibody binds not only the target protein but also other proteins. ReadyTector® reduces non-specific binding of low affinity. Therefore the additional bands are caused by real binding of the antibody. The primary antibody identifies specifically other proteins.	If possible change the antibody to solve the problem. Another possibility is to switch to the standard immunodetection protocol and to use LowCross-Buffer® as an antibody dilution buffer. LowCross-Buffer® minimizes low to medium affinity binding and reduces non-specific bands.
	The secondary antibody binds proteins of the specimen. The specimen consists of canine serum. The included secondary antibody in ReadyTector® recognizes specifically the wanted species (e.g. anti-mouse identifies mouse). But sometimes cross reactivities with rarely used species can occur. This happens with "anti-mouse" and canine serum. If you separate and blot serum from dogs, the secondary antibody included in the ReadyTector® Anti-Mouse-HRP solution detects IgG molecules of the canine serum. This leads to additional bands.	For detection of proteins in canine serum please use the standard protocol with a secondary antibody pre-adsorbed against canine. With ReadyTector® Anti-Mouse-HRP the additional bands cannot be prevented.