



Master Mixes - articles

Real-Time PCR Troubleshooting

PCR users have sometimes trouble with their real-time PCR experiments. Their real-time PCR curves give no signal or they have complication with signal in negative control. We will try to help users with the main reasons of your PCR troubles and how to solve them.

Do you have no signal?

If there is no signal in your real-time PCR results, there could be some of the following issues:

- There is no template in PCR. It looks like a trivial cause of no signal in your PCR, but always check again that you have added the template to your PCR. This can sometimes help you to save time and nerves in solving the problem.
- There are inhibitors in PCR. It is possible that your template contains inhibitors that cause poor PCR, for example salts, SDS, phenol, plant and human tissues or fluids. The solution: It is appropriate to use additives (BSA, DMSO, Betaine, etc.) to enhance your PCR. Next option is usage of internal positive control that can check the correctness of PCR.

Recommended products: gb IPC PCR Master Mix, gb OneStep IPC RT-qPCR kit

- There is problem with Probe quality or design. The probe could be partially degraded because of high amount of freeze-thaw cycles or wrong long-term storage. The probes synthesized in Generi Biotech may have up to 50 freeze-thaw cycles. It is also possible that the probe is not designed properly and doesn't bind to the amplified product. Oligos and probes
- There is an issue with thermal profile optimization. Be sure to have adequate time and temperature for all steps of your PCR.

Do you have signal in negative control?

If there is positive signal in negative control (or no template control), there could be some of the following issues:

- There is a possibility that you have added the template into your NTC reaction. Please repeat the analysis to exclude this problem.
- There is a problem with primers and probes design. It is possible that your primers and probes generate dimers and your PCR gives you false positive signal in your analysis. Try to check your PCR product by electrophoresis or by melting analysis.

Recommended products: gb SG PCR Master Mix, gb Basic PCR Master Mix

More info on website:

www.generi-biotech.com/categories/life-sciences-en/pcr-reverse-transcription-master-mixes

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