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Tips for easier switch between different PCR enzymes and mixes

Reasons to try new PCR mixes:

- You can get more value for money
- Your current reagents give low yield, high background or inconsistency
- The use of your current reagent is complicated, time and effort consuming
- You are unhappy with delivery/service

Things to check before start:

- What are the essential technical differences between your old and new PCR mix or enzyme? And how important are they?
- Will you need the same amount of the enzyme, template, and primers? For example, the amount of polymerase may vary from 0.25 to 5 units for a 50 µl reaction; use only the amounts recommended in the protocol.
- Check if the new buffer is 5X or 10X concentrated, and use it as recommended.
- Pay attention to whether the new enzyme is supplied with dNTPs in the buffer. Mix buffers well.
- Avoid using your own additives such as DMSO or BSA if the supplier does not recommend it. All additives and enhancers might already be in the supplied buffer.

Tips for correct evaluation of new PCR reagents:

1. **PROTOCOL:** Treat the new reagent according to its **specific protocol**. Read it carefully and note any differences, such as buffer concentration or pre-included dNTPs.
2. **CONTROLS:** Set up the **appropriate controls** to avoid repeating the experiment. Use a trusted control system, like pUC or lambda DNA with the right primer set, to consistently amplify in parallel within the same reaction setup.
3. **CONSISTENCY:** perform comparative experiment on the same day, in the same cycler, using the **same dilutions of the control templates** and primers; use the same pipets, water and other components. Load the same volumes on the gel for electrophoresis.
4. **MIXING:** Ensure thorough mixing of all reagents before use. Inadequate mixing of highly concentrated dNTPs or buffers can lead to inconsistent results due to concentration fluctuations.
5. **CYCLING:** Conduct an **annealing temperature gradient** with the new reagent and template/primer system, as different buffer salt concentrations affect annealing temperature requirements. Adjust **cycling times** accord to supplier's recommendations and avoid excessively long denaturation, elongation, or annealing steps.
6. **SAFETY:** Use filter-pipet tips, keep reagents on ice during reaction assembly, and adhere to standard **PCR contamination prevention** measures.

Fast & simple SOP for PCR mix comparison:

Prepare reactions, 3 repeats with controls to run the annealing temperature gradient for the new enzyme and to compare both enzymes in parallel experiment. The only difference shall be the enzyme/buffer/mix.

Reaction 1: Old reagents according to their own protocol with test primer/template

Reaction 2: New reagents exactly according to their own protocol with the same test primer/template as above

A set of controls for both old and new reagent:

Reactions 3-4: Control primer/template with the new/old enzyme – to check how they both work on your control primer/template system

Reactions 5-6: Test template added into the control system above with the new/old enzyme – to see if the test template is pure and is not used in excess (if it is not inhibiting the control PCR)

Reactions 7-8: Negative control without template, only test primer added into the control system with the new/old enzyme – to see if there is no test template cross-contamination

Reactions 9-10: With your new primer and template added into the control system above with the new/old enzyme – to see if the control system and your test system in the same tube will both work. Only if you have control system fitting into a range of the annealing temperature for the test system.

Reactions/Components	1 Old enz.	2 New enz.	3 Old enz.	4 New enz.	5 Old enz.	6 New enz.	7 Old enz.	8 New enz.	9 Old enz.	10 New enz.
Water	Use the same water for all reactions									
Buffer	Use the buffer supplied with each enzyme (not valid for Mixes)									
dNTPs	Only if needed/recommend by supplier, in recommend concentrations.									
Salts (Mg)	All these components might be included in the supplied PCR buffer!									
Additives										
Template										
Primers										
Control Templ.										
Control Primer										
Enzyme										



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professionally simple

ENZYMES MADE TO AMPLIFY

Enjoy easy-to-use, outperforming tools and consistently great results!

Why to choose highQu?

- Best value for money
- Scientific track record since 2013
- Trusted by scientists in >30 countries
- Next day delivery in Germany
- Immediate customer support

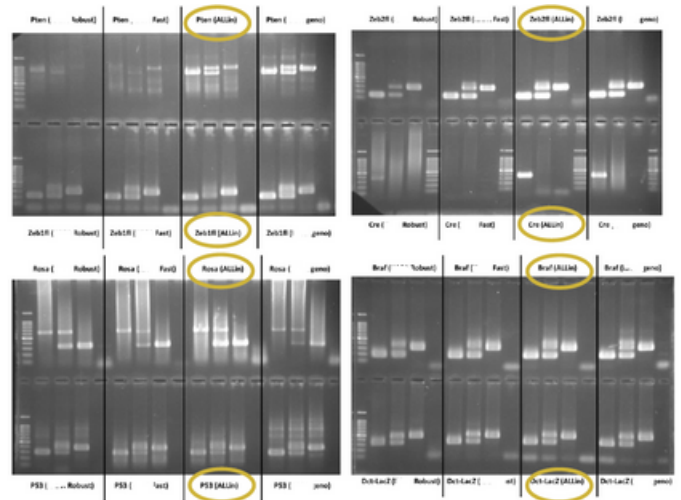
What's special about highQu PCR/qPCR reagents?

- Robust - inhibitor-resistant, minimum optimization
- Easy-to-use - simplified protocols
- Fast - designed for rapid workflows
- Outperforming – great on complex templates
- ALLin™ formulation - dNTPs in buffers, water supplied

highQu Customer's Experience: Mice Genotyping with highQu ALLin™ HS Red Taq Mastermix

The results show that highQu ALLin™ HS Red Taq Mastermix provides more reproducible results and higher specificity compared to other master mixes. 10 µl of PCR mixes (5 µl Mastermix, 0.2 µl of 25 µM primers specific to different mouse gene loci, 1 µl crude sample and 3.8 µl of home-made enhancer solution that includes betaine, DTT, DMSO and BSA) have been amplified according to cycling conditions described in the product manual.

ALLin™ HS Red Taq Mastermix is an economical high quality alternative to specialized genotyping mixes, it ensures: •best consistency •robust performance •no need for template purification •simple procedure •low background •high PCR yields •fast cycling •direct gel loading •excellent price-quality ratio



What would you like to try?

qPCR Mixes for Probes

- QPP010S ORA™ qPCR Probe Mix
- QPP040S ORA™ SEE qPCR Probe Mix
- QPP020S ORA™ qPCR Probe ROX L Mix
- QPP050S ORA™ SEE qPCR Probe ROX L Mix
- QPP030S ORA™ qPCR Probe ROX H Mix
- QPP060S ORA™ SEE qPCR Probe ROX H Mix

qPCR Mixes with Dye

- QPD010S ORA™ qPCR Green ROX L Mix
- QPD050S ORA™ SEE qPCR Green ROX L Mix
- QPD020S ORA™ qPCR Green ROX H Mix
- QPD040S ORA™ SEE qPCR Green ROX H Mix
- QPD030S ORA™ qPCR HRM Mix

One Step RT qPCR Kits for Probes

- QOP040S 4X 1Step RT qPCR Probe Kit
- QOP050S 4X 1Step RT qPCR Probe ROX L Kit
- QOP060S 4X 1Step RT qPCR Probe ROX H Kit
- QOP110S 2X 1Step RT qPCR Probe Kit
- QOP120S 2X 1Step RT qPCR Probe ROX L Kit
- QOP130S 2X 1Step RT qPCR Probe ROX H Kit

One Step RT qPCR Kits with Dye

- QOD010S 1Step RT qPCR Green ROX L Kit
- QOD020S 1Step RT qPCR Green ROX H Kit

Isothermal Amplification

- IDK0101 ALLin™ Isothermal DNA Amplification Kit
- IRK0101 ALLin™ Isothermal 1Step RNA Amplification Kit
- IDE010S phi29 DNA Polymerase
- IDE020E ALLin™ Bst DNA Polymerase
- IDE030E ALLin™ Hot Start Bst Polymerase
- IDK0201 ALLin™ HS Isothermal DNA Amplification Kit
- IDK0301 ALLin™ HS Iso-Colorimetric DNA Amplification Kit

Standard PCR

- PCE010S ALLin™ Taq DNA Polymerase
- PCM020S ALLin™ Red Taq Mastermix
- PCM010S ALLin™ Taq Mastermix
- PCE020S Taq DNA Polymerase

Hot Start PCR and Direct PCR

- HSE010S ALLin™ Hot Start Taq Polymerase
- HSM030S ALLin™ HS Red Taq Mastermix
- HSM020S ALLin™ Hot Start Taq Mastermix

High Fidelity and Long PCR

- HLE010S ALLin™ RPH Polymerase
- HLM010S ALLin™ RPH Mastermix
- HLE020S ALLin™ HiFi DNA Polymerase
- HLE030S ALLin™ Mega HiFi DNA Polymerase
- HLM020S ALLin™ Mega HiFi Mastermix
- HLM030S ALLin™ Mega HiFi Red Mastermix
- HLE040S ALLin™ Mega HS HiFi DNA Polymerase
- HLM040S ALLin™ Mega HS HiFi Mastermix
- HLM050S ALLin™ Mega HS HiFi Red Mastermix

Direct PCR

- DPK010S SampleIN™ Direct PCR Kit

PCR related Reagents & Kits

- PRK0101 Proteinase K MBG Solution
- SCR0101 Synthetic Carrier RNA
- PDK0101 PCRbeam™ Fast PCR Detection Kit
- NUM010S 25 mM dNTP Mix
- Reverse Transcription & RT-PCR**
- RTK020S 1Step RT PCR Kit
- RTK010S qScriber™ cDNA Synthesis Kit
- RTM030S HighScriber™ Reverse Transcriptase Mix
- RNI030S SecurIN™ Advanced RNase Inhibitor

Ladders for DNA Electrophoresis

- DNL010S Take5™ 1kb DNA Ladder
- DNL030S Take5™ 50 bp DNA Ladder
- DNL020S Take5™ 100 bp DNA Ladder
- DNL040S Take5™ HR DNA Ladder

Novel NA Stains for DNA Electrophoresis

- NAS030S StainIN™ eco-RED Nucleic Acid Stain
- NAS020S StainIN™ GREEN Nucleic Acid Stain

Ladders for Protein Electrophoresis

- PRL010S Cozy™ Prestained Protein Ladder
- PRL020S CozyHi™ Prestained Protein Ladder
- PRL030S CozyXL™ Prestained Protein Ladder