

Ask Frank

by Fred and Hank



FRED HYDE



HANK DAUM

Questions about EPICENTRE's Real-Time PCR Products

Q. I see EPICENTRE makes real-time PCR Kits for both SYBR® Green I dye and sequence-specific probes. When should I use SYBR Green I dye and when should I use probes?

A. The selection of which fluorescence-monitoring chemistry to use is based on a number of criteria, with both systems having several pros and cons. The use of SYBR Green I dye has the advantage of simple assay design (only two primers are needed for each target sequence and probe design is not required), and low-cost. However, its major disadvantage is its nonspecificity; it will bind to any double-stranded DNA (dsDNA). This means that both specific and nonspecific PCR products, i.e., mis-priming events and primer-dimers, will generate fluorescence. Furthermore, SYBR Green I dye cannot be used for multiplex reactions as fluorescent signals from different amplicons cannot be distinguished.

For greater detection specificity you should use a fluorescently-labeled probe that is designed to hybridize to your specific real-time PCR (qPCR) product of interest. Probe assays also allow the attachment of different fluorescent dyes to different probes, which can then be combined and used to detect more than one target in a single reaction tube (multiplex qPCR). This becomes an important factor if you have a limited amount of sample. A disadvantage with probes is that specific probes must be synthesized to detect specific sequences without primer or probe cross-hybridization; this not only requires careful design, but raises the cost of the experiment. EPICENTRE Biotechnologies' FailSafe™ GREEN and FailSafe™ PROBES Real-Time PCR Optimization Kits are designed to make the setup and optimization of SYBR Green I dye and probes-based PCR assays as rapid and as straightforward as possible.

Q. You have both TAQurate™ and FailSafe™ Real-Time PCR Systems with SYBR Green I dye and also both types of kits for probes. What is the difference and how do I decide which one to choose for real-time PCR?

A. The selection will depend on the level of "difficulty" of your qPCR reaction and, ultimately, the ability of the assay to generate highly efficient PCR amplifications (see next question). For templates that have a GC content of 50-60% and are not predicted to produce secondary structure, we recommend a TAQurate GREEN or TAQurate PROBES Real-Time PCR MasterMix. These kits use a single PCR MasterMix that will work for most templates most of the time. Simply add your primers, probe(s) and template to a tube containing the MasterMix and run the reaction in your qPCR thermal cycler.

FailSafe GREEN and FailSafe PROBES Real-Time PCR Optimizations Kits are designed for more difficult templates, such as those that may contain regions of high GC or AT content, secondary structure, or many repeats. These systems provide a set of PCR MasterMixes (12 MasterMixes in the FailSafe GREEN Kit and 8 MasterMixes in the FailSafe PROBES Kit), each of which represents one carefully selected PCR condition, and at least one of these PCR PreMixes is guaranteed to amplify the template. We highly recommend the FailSafe PROBES PCR Optimization Kit for multiplex qPCR experiments. The FailSafe System allows simple, one-step optimization of your qPCR, enabling you to achieve the best PCR efficiency over a wide dynamic range. Once you have identified which one of the FailSafe PreMixes works best for your template and primers, you simply order this PreMix by selecting either the FailSafe GREEN or FailSafe PROBES Real-Time PCR PreMix-Choice Kit knowing

that you will obtain consistently high-quality and reproducible results for the template sequence(s) tested.

Q. Why do I need to optimize my real-time PCR reactions?

A. For meaningful conclusions to be drawn from your qPCR results, it is important to optimize and validate your assays. A number of variables can affect PCR results. These include: the concentrations of primers, probes, template, magnesium chloride and thermostable DNA polymerase; template quality; sequence design of primers and probes, and the presence of PCR inhibitors.

You should aim for an amplification efficiency as close to 100% as possible. This indicates a well-optimized assay, which means the quantitative results obtained are more reliable and meaningful. Low efficiencies (<90%) may indicate poor primer/probe design, suboptimal reagent concentrations or suboptimal reaction conditions. Reaction efficiencies >110% may indicate errors in pipetting, amplification of nonspecific products such as primer-dimers, presence of PCR inhibitors or poor template quality. By properly optimizing your PCR assay you will enable a wide dynamic range allowing the quantitation of both high- and low- expressing genes. The use of EPICENTRE's FailSafe Real-Time PCR Optimization Kits makes the process of successful qPCR optimization much easier.

Q. Will the FailSafe and TAQurate Real-Time PCR Kits work with all real-time PCR instruments?

A. Yes. A stabilizer reagent and passive reference dye are included in both types of kits so that they can also be used on instrument platforms that require them.