Resource Summary Report

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How to Setup and Perform a qPCR Experiment.

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Protocol Information

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Authors: Promega, Trevor Wagner

Group: Promega

Summary: GoTaq® qPCR Master Mix(a,b) is a reagent system for quantitative PCR (qPCR). The system contains a new fluorescent DNA-binding dye that often exhibits greater fluorescence enhancement upon binding to double-stranded DNA (dsDNA) than SYBR. Green I.GoTaq® qPCR Master Mix is provided as a simple-to-use, stabilized 2X formulation that includes all components for qPCR except sample DNA, primers and water. This formulation, which includes a proprietary dsDNA-binding dye, a low level of carboxy-X-rhodamine (CXR) reference dye (identical to ROX™ dye), GoTaq. Hot Start Polymerase, MgCl2, dNTPs and a proprietary reaction buffer, produces optimal results in qPCR experiments. A separate tube of CXR Reference Dye is included for use with instruments that require a higher level of reference dye than that in the GoTaq® qPCR Master Mix.Advantages of the GoTaq® qPCR Master MixDye: The proprietary dye provides brighter dsDNA-dependent fluorescence than SYBR. Green I, with less PCR inhibition than SYBR® Green. The dye enables efficient amplification, resulting in earlier quantification cycle (Cq) values and an expanded linear range using the same filters and settings as SYBR® Green I. The CXR reference dye can be detected using the same filters and settings as those used for ROX™ dye.Quantification cycle is formerly known as cycle threshold (Ct).Polymerase/Buffer Formulation: GoTaq® Hot Start Polymerase contains full-length Taq DNA polymerase bound to a proprietary antibody that prevents polymerase activity at room temperature. Thermal activation is achieved by incubating the assembled reaction at 95°C for 2 minutes. The proprietary polymerase/buffer formulation accommodates extended cycle numbers (45–50 cycles) and is compatible with thermal cycling programs that require extended activation (95°C for 10 minutes).Performance: You can expect reliable performance with minimal lot-to-lot variation: efficient, sensitive and linear qPCR amplification over a wide dynamic range.GoTaq® qPCR Master Mix ProtocolIf you are currently performing dye-based qPCR, the GoTaq® qPCR Master Mix

can simply be substituted for your current master mix. For consistency within an experimental set, prepare a sufficient volume of reaction mix without template DNA for the DNA standard reactions and experimental sample reactions. The protocol for a 50?l reaction is outlined below. Component volumes may be scaled as appropriate. This protocol assumes that 20% of the reaction volume is DNA template (e.g., 10?l of DNA template added to 40?l of reaction mix). If the volume of DNA template is more or less than 10?l, adjust the volume of Nuclease-Free Water accordingly so that the final reaction volume is 50?l.

Affiliations: , Promega Corporation

External URL: https://www.promega.com/-/media/files/resources/protocols/technical-manuals/101/gotaq-qpcr-master-mix-protocol.pdf?la=en

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