

iQ™ Multiplex Powermix

50 x 50 µl reactions	172-5848
200 x 50 µl reactions	172-5849

For research purposes only
Store at –20°C

Storage and Stability

The iQ Multiplex Powermix is stable for 1 year when stored in a constant temperature freezer at –20°C. For convenience, it may be stored unfrozen for up to one month. After thawing, mix thoroughly, then centrifuge briefly to collect tube contents before using. Repeated freezing and thawing of the iQ Multiplex Powermix is not recommended.

Kit Contents

The iQ Multiplex Powermix is a 2X concentrated, ready-to-use reaction cocktail containing all components, except primers, probe and template for real-time quantitative PCR (qPCR). The mix has been optimized to deliver maximum PCR efficiency, sensitivity and precision in multiplex qPCR applications. A common phenomenon of multiplex PCR is dominance of the high copy target(s) in the amplification. Often this skews, or masks, representation and quantification of lower copy target sequences. iQ Multiplex Powermix delivers dynamic range and sensitivity that is comparable to singleplex qPCR probe assays without the need for limiting or variable primer concentration(s).

The antibody-mediated automatic hot-start employed by iTaq™ DNA polymerase sequesters polymerase activity prior to the initial PCR denaturation step. Upon heat activation (2 min at 95°C), the antibodies denature irreversibly, releasing fully active and unmodified iTaq DNA polymerase. This enables specific and efficient primer extension with the convenience of room temperature reaction assembly.

Reagent

Description

iQ Multiplex Powermix (2X)	2X reaction buffer with dNTPs 12 mM MgCl ₂ iTag DNA polymerase, and stabilizers
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Reaction Set Up

Thaw all components at room temperature. Mix vigorously, then centrifuge to collect contents to the bottom of the tube before using.

Component	Volume for 50 µl rxn	Final Concentration
iQ Multiplex Powermix (2X)	25 µl	1X
Forward primer(s)	Variable	300 nM
Reverse primer(s)	Variable	300 nM
Probe(s)	Variable	200 nM
DNA templates	Variable	
<u>RNase/DNase-free water</u>	<u>Variable</u>	
Final Volume (µl)	50 µl	

Recommendations for Optimal Results using the iQ Multiplex Powermix:

- Preparation of a reaction cocktail is crucial in quantitative PCR applications to reduce pipetting errors and maximize assay precision and accuracy. Assemble the reaction cocktail with all required components except sample template (genomic DNA or cDNA) and dispense equal aliquots into each reaction tube. Add target sample to each reaction as the final step. Addition of sample as 5–10 μ l volumes will improve assay precision. Replicate samples should be assembled as a master mix with a single addition of sample template.
- Primer and probe design is critical for successful multiplex qPCR. In addition to following the general guidelines for probe-based qPCR assays, it is important that each primer and probe have similar thermodynamic properties and balanced T_m s to allow efficient PCR amplification using a fixed temperature cycling regimen. Cross hybridization between all primers in the multiplex reaction must be minimized. The use of software design tools, such as Beacon Designer often facilitates the design of effective multiplex primer/probe sets. Each probe for a multiplex assay should be labeled using dyes having discrete fluorescent excitation and emission optima.
- Use equal concentration of each primer. 300 nM is effective for most primer sets. Limiting primer optimization for high copy reference genes is not required.
- Suggested input quantities of template are:
 - cDNA from 1 μ g to 1 μ g of total RNA
 - 100 pg to 1 μ g of genomic DNA
- Gently mix and ensure that all components are at the bottom of the amplification tube. Centrifuge briefly if needed.
- Full activation of iTaq DNA polymerase occurs within 30 seconds at 95°C. Initial denaturation times greater than 3 minutes are not recommended.
- Suggested cycling conditions:

Initial denaturation	2–3 min at 95°C,
PCR cycling (30–45 cycles)	10 to 15 s at 95°C,
	45 to 60 s at 55–60°C (collect and analyze data)

Reagents and Materials Not Supplied

Gene-specific primers and probes

Pipette tips, aerosol barrier tips, such as:

The Xcluda® Style B, 211-2006

Nuclease-free tubes or plates, such as:

0.2 ml Thin-Wall Tubes, 223-9473 or plates, 223-9441

RNA purification kit, such as:

Aurum™ Total RNA Mini Kit, 732-6820, or

Aurum Total RNA Kit, 2 x 96 well, 732-6800

cDNA Synthesis kits, such as:

iScript™ cDNA Synthesis Kit, 170-8891, or

iScript Select cDNA Synthesis kit, 170-8897

To learn more about Bio-Rad's complete solution for Amplification, visit our website:

www.bio-rad.com/amplification

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A license to perform the patented 5' Nuclease Process for research is obtained by the purchase of (i) both Authorized 5' Nuclease Core Kit and Licensed Probe, (ii) a Licensed 5' Nuclease Kit, or (iii) license rights from Applied Biosystems.

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