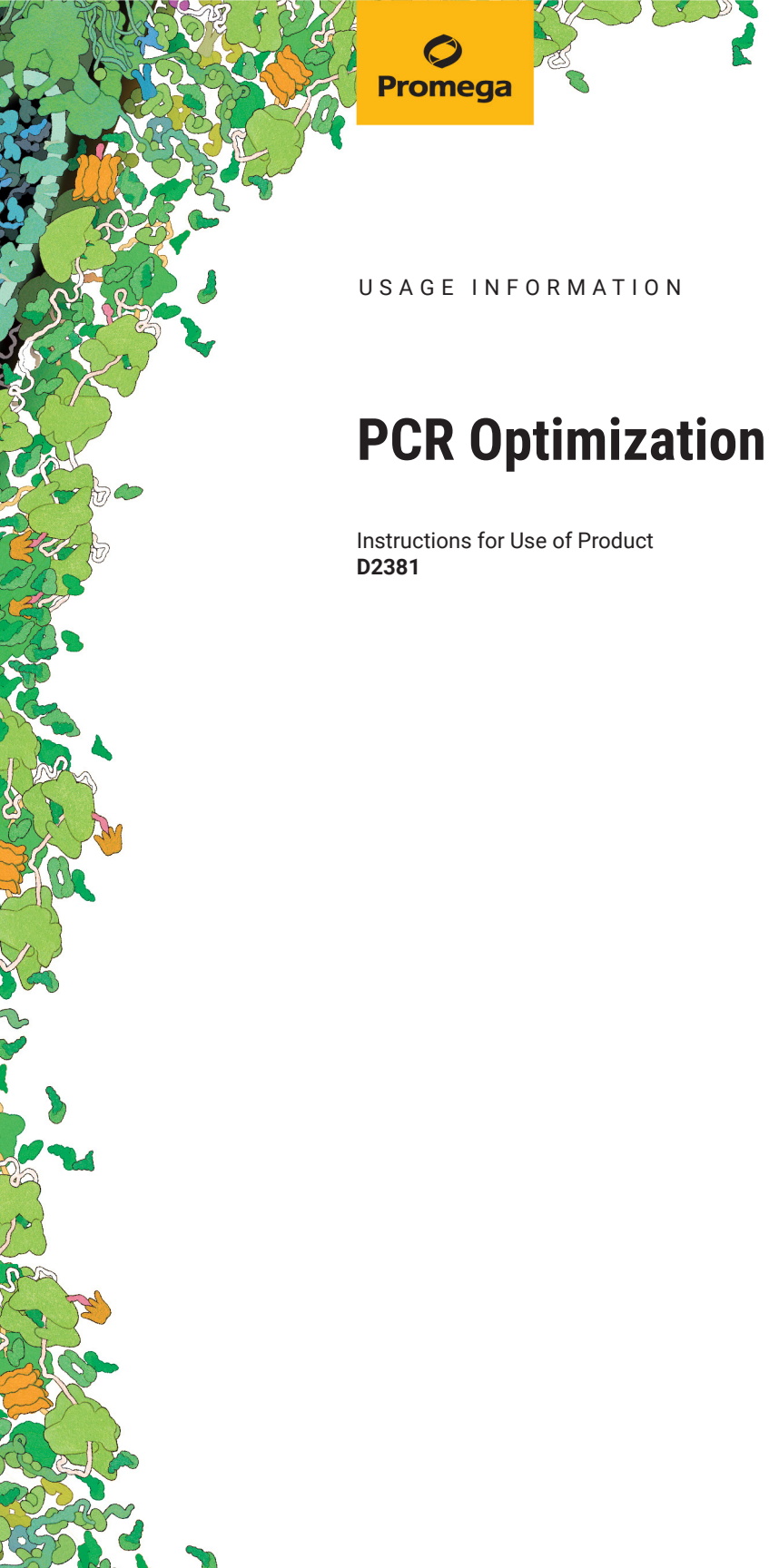

USAGE INFORMATION

PCR Optimization Kit

Instructions for Use of Product
D2381

Revised 11/25
TM497

PCR Optimization Kit

All technical literature is available at: www.promega.com/protocols/
Visit the website to verify that you are using the most current version of this Technical Manual.
Email Promega Technical Services if you have questions on use of this system: techserv@promega.com

1. Description

The PCR Optimization Kit contains pre-formulated PCR master mix components used to perform a short series of amplification experiments with user-provided primers/probes to survey and quickly identify the optimal PCR amplification reaction conditions specific to the user's application. The PCR Optimization kit facilitates the rapid identification of a personalized, made-to-order, PCR Master Mix formulation that can then be ordered from Promega.

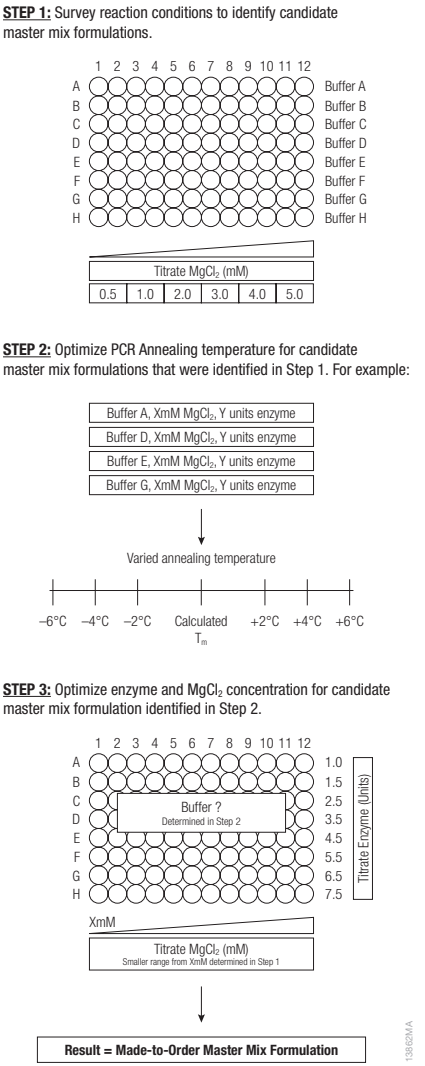

Figure 1. Example of PCR optimization strategy using the PCR Optimization Kit.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
PCR Optimization Kit	**1 each**	**D2381**

For Laboratory Use. Includes:

- 1ml 5X PCR Buffer A
- 1ml 5X PCR Buffer B
- 1ml 5X PCR Buffer C
- 1ml 5X PCR Buffer D
- 1ml 5X PCR Buffer E
- 1ml 5X PCR Buffer F
- 1ml 5X PCR Buffer G
- 1ml 5X PCR Buffer H
- 1.2ml 25mM $MgCl_2$ Solution
- 500units GoTaq® MDx Hot Start Polymerase

Storage Conditions: Store the PCR Optimization Kit at –30°C to –10°C.

3. General Guidelines for Amplification by PCR

Materials to Be Supplied by User

- sterile, aerosol-resistant pipette tips
- nuclease-free pipettors dedicated to pre-amplification work
- 1.5ml microcentrifuge tubes
- nuclease-free water
- PCR primers and probes
- template DNA

Thaw the 5X Buffers and $MgCl_2$ Solution at room temperature. Briefly vortex the 5X PCR Buffers and $MgCl_2$ Solution for 3–5 seconds to mix before use.

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3.A. Step 1: Identifying Optimal PCR Buffer Formulation and $MgCl_2$ Concentrations

This is an example of an initial experiment to survey the performance of all buffer formulations (A–H) and a range of $MgCl_2$ concentrations to identify candidate master mix formulation(s) that work optimally for your specific assay. The reagent composition for a single well, 20µl reaction is shown. Component volumes may be scaled for larger or smaller reaction volumes. If performing probe-based amplification reactions, the reaction concentrations for primers and probes should be optimized for each primer/probe combination.

Component	Concentration	Volume	Final Concentration
PCR Buffer (A–H)	5X	4µl	1X
$MgCl_2$ Solution*	25mM	0.8–3.2µl	1–4mM*
Upstream Primer	20X	1µl	0.1–1µM
Downstream Primer	20X	1µl	0.1–1µM
Hydrolysis Probe (if applicable)	20X	0.5µl	0.1–0.5µM
GoTaq® MDx Hot Start Polymerase	>8u/µl**	0.25µl	1.25U*
Template DNA		Xµl	<0.5µg/µl
Nuclease-Free Water to 20µl		Yµl	

* Optimization of $MgCl_2$ and enzyme concentration specific to the assay is strongly recommended.
** Enzyme concentrations may vary. Enzyme concentration is indicated on the product label.

Thermal Cycling

The cycling parameters below are offered as a guideline and may require modification for optimal results.

	Standard Cycling Conditions			FAST Cycling Conditions		
Step	**Cycles**	**Temperature**	**Time**	**Cycles**	**Temperature**	**Time**
Activate Enzyme*	1	95°C	2 minutes	1	95°C	2 minutes
Denature	40	95°C	15 seconds	40	95°C	3 seconds
Anneal/ Extend**		60°C	1 minute		60°C	30 seconds

*An initial 2-minute denaturation step is required to inactivate the antibody and initiate hot-start PCR.
**The optimal annealing temperature in PCR amplification is dependent on the reaction buffer conditions. Optimization of annealing temperature may be required.

3.B. Step 2: Identifying Optimal Annealing Temperature for Candidate Master Mix Formulations

The optimal annealing temperature in PCR amplification is dependent upon the reaction buffer composition. Optimization of the annealing temperature for the reaction buffer used in the PCR amplification may be required and is recommended to achieve optimal amplification performance.

The results from Step 1 identified one or multiple candidate master mix formulations that work better than others for your PCR assay(s). Using these candidate master mix formulations, optimize the annealing temperature by performing amplification reactions at increments of 2°C, 4°C and 6°C above and below the calculated annealing temperature.

3.C. Step 3: Identifying Optimal Enzyme Concentration for Candidate Master Mix Formulations

The optimal concentration of enzyme is dependent upon the reaction buffer composition. The optimal $MgCl_2$ concentration is dependent on the enzyme concentration in the reaction. Therefore optimization of the enzyme concentration for the reaction buffer used in the PCR amplification, and further refinement of the $MgCl_2$ concentration is recommended to achieve optimal performance.

The results from Step 2 identified a primary buffer formulation and optimal annealing conditions for your PCR. Using the candidate buffer formulation, optimize the units of enzyme and further refine the $MgCl_2$ concentration in the PCR. The enzyme concentration should be titrated until either the PCR amplification fails or suboptimal performance occurs.

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4. Additional Guidelines

4.A. qPCR Primers and Probes

The concentrations of primers and probes should be optimized for each primer/probe combination. For gene expression assays, primer and probe concentration may need to be adjusted based on target abundance. As a general rule, a concentration of 900nM for PCR primers and 250nM for the hydrolysis probe is recommended as a starting point. Concentrations of PCR primers may range from 200nM to 1µM, while probe concentration may range from 100nM to 200nM; titrations should be performed to ensure optimal results. We recommend preparing and storing 20X solutions of the primers and hydrolysis probe. The primers and probe(s) should be stored separately from the enzyme until final reaction assembly.

4.B. Genomic DNA Template Quantity

We recommend using ≤250ng of genomic DNA for a template.

4.C. Lyophilization Compatibility

The 5X PCR Buffers (B, C, D, E and H) are lyophilization-compatible.

4.D. CXR Reference Dye

The 5X PCR Buffers do not contain a reference dye; however a separate tube of carboxy-X-rhodamine (CXR) reference dye can be added to the buffer. (Cat.# C5411). Addition of the reference dye will help maximize effectiveness of a master mix when used on real-time PCR instruments that allow normalization. The CXR reference dye has the same spectral properties as the ROX™ dye. The dye is provided at a concentration of 30µM.

Some instrumentation is designed to normalize with a low concentration of ROX™ reference dye and we recommend that the CXR reference dye be added to a final reaction concentration of 30nM for instruments that recommend a "low" level of ROX™ dye. Other instruments require ROX™ at a high concentration for normalization and we recommend that the CXR reference dye be added to a final reaction concentration of 500nM for those instruments.

- For "Low Dye" Instruments: Add 5µl to 1ml of each 5X PCR Buffer before use.
- For "High Dye" Instruments: Add 83µl to 1ml of each 5X PCR Buffer before use.

For a list of "Low Dye" and "High Dye" instruments, please refer to a list provided in the *GoTaq® Probe qPCR Master Mix Technical Manual*, TM378, which can be found at **www.promega.com/protocols.**

5. Ordering Promega Made-to-Order 2X PCR Master Mix

Once you have identified your reaction's optimal formulation, you may wish to continue your work with 2X PCR Master Mix that is custom made for you under cGMP. Provide us with the volume of the PCR components in your reaction (5X Buffer, $MgCl_2$ and enzyme) and a customized 2X PCR Master Mix to your optimized formulation will be manufactured under cGMP. To place your order or for more information, please visit:
www.promega.com/c/global/forms/lets-talk-custom-form/ or contact your local Promega representative.

6. Summary of Changes

The following changes were made to the 11/25 revision of this document:

1. The cover image and fonts were updated.
2. A typo was corrected and minor text edits made.
3. A third party trademark was updated.
4. Updated the custom order url in Section 5.