ChoiceTaq™ Blue Mastermix

Shipping: On Dry Ice
Batch No.: See Vial
Concentration: 25µl/reaction

Catalog Numbers
CB4065-7 (100 reactions)
CB4065-8 (400 reactions)

Store at –20°C

Caution: Do not subject to repeated freeze/thaw cycles.

Storage and stability:
The ChoiceTaq™ Blue Mastermix is shipped on Dry Ice and can be stored for up to 12 months at -20°C.
Repeated freeze/thaw cycles should be avoided.

Safety precautions:
Harmful if swallowed. Irritating to eyes, respiratory system and skin. Please refer to the material safety data sheet for further information.

Unit definition:
One unit is defined as the amount of enzyme that incorporates 10nmoles of dNTPs into acid-insoluble form in 30 minutes at 72°C.

Notes:
Research use only.

Description

ChoiceTaq™ Blue Mastermix is a complete "ready-to-go" 2X reaction mix which only requires the users to add water, template and primers. The product is supplied with Blue Taq DNA polymerase, dNTP mix, and PCR reaction buffer containing $MgCl_2$, which produces **a final Mg^{2+} concentration of 1.5mM**. Blue Taq differs from regular Taq in that it contains blue dye for direct gel loading after PCR reaction with no need of any loading buffer. It is ideal for primary extension reaction with DNA fragments having dA overhang on 3' ends; at extension speed of 1200 bases per minute and optimized between 65-75°C.

Components

	100 reactions	400 reactions
ChoiceTaq Blue Mastermix	2 x 1.25ml	8 x 1.25ml

Standard ChoiceTaq™ Blue Mastermix Protocol

The following protocol is for a standard 50µl reaction and can be used as a starting point for reaction optimization.

PCR reaction set-up:
All reactions must be set-up on ice.

ChoiceTaq™ Blue Mastermix	25µl
Template	as required
Primers (50µM each)	0.5µl each
Water (ddH_2O)	bring to 50µl

PCR cycling conditions
We suggest these conditions in the first instance:

Step	Temperature	Time	Cycles
Initial denaturation	94°C	1-3min	1
Denaturation	94°C	45s	25-35
Annealing	56°C	30s	
Extension	72°C	1-2min	
Post elongation	72°C	10min	1

Important considerations and PCR optimization

The optimal conditions will vary from reaction to reaction and are dependent on the template/primers used.

2x ChoiceTaq Blue Mastermix: 2.5U of Blue Taq DNA polymerase, 20mM Tris-HCl (pH 9.0), 3mM $MgCl_2$, 20mM KCl, 16mM $(NH_4)_2SO_4$, 0.1% NP-40, 1.6mM dNTP mix. Buffer produces a final Mg^{2+} concentration of 1.5mM.

Primers: Forward and reverse primers are generally used at the final concentration of 0.2-0.6µM each. As a starting point, we recommend using 0.5µM as a final concentration of each primer. Too high a primer concentration can reduce the specificity of priming, resulting in non-specific products.

Template: The amount of template in the reaction depends mainly on the type of DNA used. For templates with low structural complexity, such as plasmid DNA, we recommend using 50pg-10ng DNA per 50µl reaction volume. For eukaryotic genomic DNA, we recommend a starting amount of 200ng DNA per 50µl reaction; this can be varied between 5ng-500ng. It is important to avoid using template re-suspended in EDTA-containing solutions (*e.g.* TE buffer) since EDTA chelates free Mg^{2+}.

Initial Denaturation: An initial denaturation step of 1min at 94°C is recommended for non-complex templates like plasmid DNA or cDNA. For more complex templates such as eukaryotic genomic DNA, longer initial denaturation times of up to 3mins are required in order to facilitate complete melting of the DNA.

Denaturation: Our protocol recommends a 45s cycling denaturation step at 94°C which is also suited to GC-rich templates, for low GC content (40-45%) templates, the denaturation time can be decreased.

Annealing temperature and time: The optimal annealing temperature is dependent upon the primer sequences and is usually 2-5°C below the lower Tm of the pair. We recommend running a temperature gradient to determine the optimal annealing temperature. Alternatively, 56°C can be used as a starting point. Depending on the reaction, the annealing time can also be adjusted.

Extension: Extension time is one to two minutes depending on the length of templates. Both time and temperature can be adjusted based on the sequence of PCR products.

Phone: 800-453-0385
Fax: 908-757-7551
P.O. Box 4588, Metuchen, NJ 08840