

Robust Ready™ PCR Master Mix-Dye

(Cat# M1131-200, -1000; 2X PCR Master Mix with dye; Store at -20°C)

I. Introduction:

Robust Ready™ 2X PCR Master Mix (with Dye) is a ready-to-use mixture of high-quality Robust Ready™ Taq DNA Polymerase, deoxynucleotides, and reaction buffer in a 2X concentration. It contains all the necessary reagents for amplification of DNA. To set up a PCR reaction: add DNA template, primers and water. PCR products, amplified up to 6 kb in length with Robust Ready™ Taq DNA Polymerase, contain a mixture of blunt ends and single base (A) 3' overhang. The error rate of this PCR amplification is 7.5×10^{-6} per nucleotide per cycle. The products can be used for direct T/A cloning, but its efficiency is not as high as PCR products amplified with Taq DNA Polymerase alone. Robust Ready™ Master Mix-Dye includes an inert blue dye and a stabilizer which allow for direct loading of the PCR product(s) onto an agarose gel.



II. Application:

- Routine PCR amplification of DNA templates up to 6 kb
- Suitable for a wide range of PCR assays
- TA cloning

III. Key Features:

- Saves preparation time by combining Robust Ready™ Taq DNA Polymerase, dNTPs and reaction buffer in a ready-to-use mixture
- Reduces the risk of contamination by decreasing the number of pipetting steps
- Provides consistent reaction performance and results
- Improved sensitivity and fidelity as compared to the conventional Taq DNA Polymerase
- Robust and consistent performance across a wide range of templates
- An economical alternative to Taq DNA Polymerase

IV. Package Contents:

Cat. No.	Quantity	Form
M1131-200	5.0 ml (200 Rxns)	5 X 1.0 ml vials
M1131-1000	25.0 ml (1000 Rxns)	25 X 1.0 ml vials

V. User Supplied Reagents and Equipment:

- PCR Tubes
- PCR Instrument
- Pipettes
- Water, Nuclease-free
- Primers (forward and reverse)
- Template DNA

VI. Shipment and Storage:

Upon arrival, the Robust Ready™ 2X Master Mix should be stored at -20°C. 2X PCR Taq Master Mix-Dye are stable at 4°C for three months or for fifteen freeze-thaw cycles. For daily use, we recommend keeping an aliquot at 4°C. The Robust Ready™ 2X Master Mix is stable for 1 year from the date of shipping when stored and handled properly. Briefly centrifuge small vials prior to opening.

VII. PCR Protocol:

1. All PCR experiments should be assembled in a nuclease-free environment. In addition, DNA sample preparation, reaction set-up and subsequent reaction(s) should be performed in separate areas to avoid cross-contamination. The use of "clean" pipettors designated for PCR and aerosol-resistant barrier tips are recommended. Always keep the control DNA and other templates to be amplified isolated from the other components.
2. A negative control reaction (omitting template DNA) should always be performed in tandem with sample PCR to confirm the absence of DNA contamination.
3. Add the following components to a sterile 0.2 ml PCR tube sitting on ice.

Components	Volume	Final concentration
Robust Ready™ PCR Mix-Dye	25 µl	1X
10 µM Forward Primer	1-2.5 µl	200-500 nM
10 µM Reverse Primer	1-2.5 µl	200-500 nM
Template DNA	~100 ng	~2 ng/ µl
Water, Nuclease-free	Up to 50 µl	-

We recommend preparing a master mix for multiple reactions to minimize reagent loss and enable accurate pipetting.

4. Mix contents of tube and centrifuge briefly.
5. Incubate tube in a thermal cycler at 94°C for 3 mins to completely denature the template.
6. Perform 30-35 cycles of PCR amplification as follows:
Denature: 94°C for 30 secs; Anneal: 45 - 72°C for 30 secs; Extend: 72°C for 1 min/1 kb template

7. Incubate for an additional 5 mins at 72°C and maintain the reaction at 4°C. The samples can be stored at -20°C until use.
8. Analyze the amplification products by agarose gel electrophoresis and visualize by ethidium bromide or other DNA staining. Use appropriate molecular weight standards.

VIII. Related Products:

BV Product Name	BV Cat. No.
M1127-200	Ready™ PCR Master Mix
M1127-1000	Ready™ PCR Master Mix
M1128-200	Ready™ PCR Master Mix-Dye
M1128-1000	Ready™ PCR Master Mix-Dye
M1129-200	Image Ready™ PCR Master Mix
M1130-200	Robust Ready™ PCR Master Mix
M1130-1000	Robust Ready™ PCR Master Mix
M1131-200	Robust Ready™ PCR Master Mix-Dye
M1131-1000	Robust Ready™ PCR Master Mix-Dye
M1132-200	Rigor™ PCR Master Mix
M1133-200	Rigor™ PCR Master Mix-Dye
M1134-200	Breeze™ PCR Master Mix
M1135-200	Breeze™ PCR Master Mix-Dye
M1136-200	Distant™ PCR Master Mix
M1137-200	Distant™ PCR Master Mix-Dye
M1138-200	Image Distant™ PCR Master Mix
M1139-200	Advance™ PCR Master Mix
M1140-200	Advance™ PCR Master Mix-Dye
M1141-200	Fire Start™ PCR Master Mix
M1142-200	Fire Start™ PCR Master Mix-Dye
M1143-200	Whole Blood PCR Master Mix
M1144-25	Plant Advance™ PCR Kit
M1145-100	Tissue Advance™ PCR Kit
M1146- M1153	DNA Polymerases

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