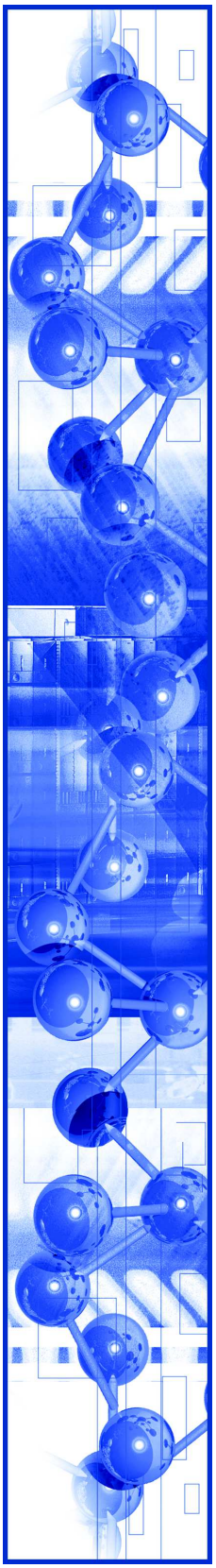

Lyophilized Ready PCR Mix, 2X

Code	Description	Size
1B1388-2VL	Lyophilized Ready PCR Mix, 2X *Includes:* 2 Vials Lyophilized Ready PCR Mix, 2X Lyophilized Ready PCR Mix includes all reagents except dissolving water, template and primer DNA	2 Vials of 2X reaction mix (100 Rxn)
1B1388-1VL	Lyophilized Ready PCR Mix, 2X *Includes:* 1 Vial Lyophilized Ready PCR Mix, 2X Lyophilized Ready PCR Mix includes all reagents except dissolving water, template and primer DNA	1 Vial of 2X reaction mix (50 Rxn)

General Information:

Lyophilized Ready PCR Mix, 2X, offers the same conveniences of our Ready PCR Mix, 2X, but in a lyophilized form, allowing greater stability. The lyophilized formulation allows room temperature storage until dissolution with water. All components for assembly and performance of PCR reactions as well as loading and visualization of PCR products on agarose gels are included. The user supplies high purity grade water, primer and template DNA.

Lyophilized Ready PCR Mix is supplied as a 2X powder of reaction buffer, AMRESCO's Extender™ Taq polymerase blend, dNTPs, electrophoresis tracking dye and a non-mutagenic EZ-Vision visualization dye. The powder can be stored at room temperature prior to dissolving in water. Once dissolved, the reaction mix must be stored frozen. Similar to AMRESCO's Ready PCR Mix, when amplification is complete, the PCR reaction can be directly loaded and separated on an agarose gel using the magenta-colored tracking dye (migrating at approximately 10bp on a 1% gel) to monitor the migration. After electrophoresis, the PCR products are immediately visualized with standard UV illumination without additional post-run staining and destaining steps.

Storage/Stability:

Store at 18-26°C as a lyophilized powder.
Once dissolved, store at -20℃. Ready PCR Mix, 2X i s stable through 15 freeze-thaw cycles.

Application Disclaimer

For research use only.
Not for therapeutic or diagnostic use.

Procedure:

Resuspend Lyophilized Sample:

1. Add 1.20 mL of high purity, nuclease-free water (E476) to one vial of Lyophilized Ready Mix, 2X.
2. Mix until all solid is dissolved by gently pipetting mixture 3-4 times.
3. Store on ice or frozen until ready to use. This mix can be subsequently used for standard PCR or colony screening.

➥*If storing frozen, transfer to plastic, sterile 1.7mL microcentrifuge tube.*

Standard PCR Reactions:

The following protocol applies to single reactions where only primers, template, and water need to be added.

4. Thaw primers, template DNA, and dissolved Lyophilized Ready Master Mix and place on ice.
5. Assemble reactions on ice according to table below:

Components	Volume (50 µL Reaction)	Final Concentration
Dissolved Lyophilized Ready PCR Mix, 2X	25 µL	1X
25 µM fwd primer	0.5–2.0 µL	0.25–1.0 µM
25 µM rev primer	0.5–2.0 µL	0.25–1.0 µM
5 ng/µL Template	0.2–10 µL	1 – 50 ng
Nuclease-Free Water	As needed	–

6. Perform standard PCR amplification. *Example:*

Steps	Time (minutes)	Temperature (℃)
A	2:00	95
B	0:30	95
C	0:30	55 – 65
D	1:00*	68 – 72
Repeat Steps B – D 29 times		
E	7:00	68
F	Hold	4

***** *1 minute for every 1 KB of expected PCR product size.*

7. Load and separate PCR products on an agarose gel at 5 – 8 V/cm. PCR products can be visualized with a standard ultra-violet light source. Fluorescent dye emits in the blue spectra for documentation.

Colony Screening Reactions:

4. Thaw primers and dissolved Lyophilized Ready Master Mix and place on ice. One primer should be complementary to the insert and the other should be complementary to the plasmid.
5. Assemble desired number of reactions on ice according to the table below.

Components	Volume (50µL Reaction)	Final Concentration
Dissolved Lyophilized Ready PCR Mix, 2X	25 µL	1X
25 µM fwd primer	0.5–2.0 µL	0.25–1.0 µM
25 µM rev primer	0.5–2.0 µL	0.25–1.0 µM
Nuclease-Free Water	As needed	–

6. Pick and resuspend a colony in the PCR reaction.
7. Remove 5 µL from the PCR reaction and place in 200 µL of LB and antibiotic in a well from a 96-well plate.

➥*To insure correct identification of positive colonies, numbering should be consistent between the PCR reaction tube or well, and wells in the plate for colony growth.*

8. When a sufficient number of colonies have been selected, place the 96-well plate at 37 ℃ for ~8 h ours.
9. Perform PCR amplification.

Example:

Steps	Time (minutes)	Temp. (℃)
A	5:00	95
B	0:30	95
C	0:30	55 – 65
D	1:00*	68 – 72
Repeat Steps B – D 29 times		
E	7:00	68
F	Hold	4

**Time should be 1 minute for every 1 KB of expected PCR product size*.

10. Load and separate PCR products on an agarose gel at 5 – 8 V/cm. PCR products can be visualized with a standard ultra-violet light source. Fluorescent dye emits in the blue spectra for documentation.

Frequently Asked Questions

Questions	Answers
Why does my sample float out of the wells when loading onto gel?	1. Reactions intended to be used with agarose gels in TAE buffer. Sample density is too light. Add glycerol to sample and reload.
Why don't I see PCR product on my gel?	1. No DNA-Not enough DNA loaded. Load more sample. 2. No DNA. PCR reaction did not work. Try modifying PCR cycle parameters.
Why do I see multiple or unexpected product sizes?	1. Contamination of another target DNA. 2. Non-specific primer annealing. 3. Primer-dimers.

Related Products

Code	Product
E476	Water, Sterile, Nuclease-Free
Agarose	
0710-500G	Agarose I™, 500 g General Use (also available as tablets, K857-100TABS)
J234-250G	Agarose SFR™ Super Fine Resolution for superior resolution of nucleic acid fragments between 200 and 1,000 base pairs.
E776-100G	Agarose 3:1 HRB™ High Resolution Blend for resolution of nucleic acid fragments below 1,000 base pairs.
Buffers	
0658-4L	TBE Buffer, 10X Liquid Concentrate
0478-2PK	TBE Buffer, 10X Ready-Pack™
0796-1.6L	TAE Buffer, 25X Liquid Concentrate
Markers	
K811-50RXN	PCR DNA Marker™ 8 bands ranging from 50 to 2000 base pairs Supplied ready-to-use in loading buffer
E854-100RXN	PCR DNA Marker™ 8 bands ranging from 50 to 2000 base pairs
N746-100RXN	Ready Ladder, 50bp DNA Marker 10 fragments ranging from 50 to 500 base pairs Supplied ready-to-use in loading buffer

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