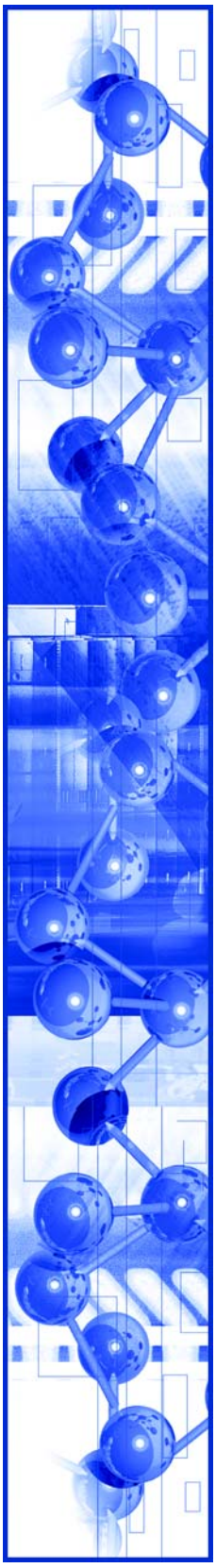

Ready PCR Mix, 2X

Code	Description	Size
N806-2x1.25ML	Ready PCR Mix, 2X *Includes*: 2 x 1.25 ml Ready PCR Mix, 2X Ready PCR Mix includes all reagents except template and primer DNA	2x1.25 ml tubes of 2X reaction mix

General Information

Ready PCR Mix, 2X, offers a single-step procedure for performing PCR reactions followed by analysis on agarose gels without addition of loading buffer. 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 primer and template DNA.

Ready PCR Mix is supplied as a 2X mixture of reaction buffer, AMRESCO's Extender™ Taq polymerase blend, dNTPs, electrophoresis tracking dye and a non-mutagenic EZ-Vision visualization dye. Once 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 electrophoreses the PCR products are immediately visualized with standard UV illumination without additional post-run staining and destaining steps.

Storage/Stability:

Store at -20°C. Ready PCR Mix, 2X is stable through 15 freeze-thaw cycles.

Application Disclaimer

For Research Use Only.
Not for Therapeutic or Diagnostic Use.

Procedure:

Standard PCR Reactions:

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

1. Thaw primers, template DNA, and Ready Master Mix and place on ice.
2. Assemble reactions on ice according to the following table:

Components	Volume (50 µL Reaction)	Final Concentration
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	–

3. Perform standard PCR amplification

Example:

Steps	Time (minutes)	Temperature (°C)
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

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

4. 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

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

Components	Volume (50µL Reaction)	Final Concentration
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	–

3. Pick and resuspend a colony in the PCR reaction.
4. 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 PCR reaction tube or well, and wells in the plate for colony growth.*

5. When a sufficient number of colonies have been selected, place the 96-well plate at 37 °C for ~8 hours.
6. Perform PCR amplification.

Example:

Steps	Time (minutes)	Temp. (°C)
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*.

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.

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

Visit the AMRESCO website to view additional related products

www.amresco-inc.com

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