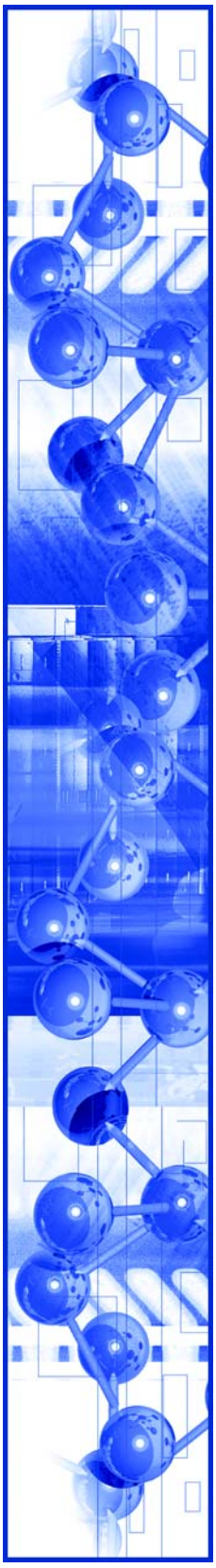

Extender PCR-to-Gel Master Mix, 2X

Code	Description	Size
N867-2x1.25ML	Extender PCR-to-Gel Master Mix, 2X *Includes*: PCR reaction reagents Gel loading buffer/tracking dye	2x1.25 ml tubes of 2X reaction mix
N867-1.25ML		1x1.25 ml tubes of 2X reaction mix

General Information

Extender PCR-to-Gel Master Mix, 2X, is a single solution for performing PCR reactions and analysis of reaction products on agarose gels. All components for assembly of PCR reactions (except templates and primers) as well as loading and tracking of PCR products on agarose gels are included. The user supplies primer and template DNA.

Extender PCR-to-Gel Master Mix, is supplied as a 2X mixture of reaction buffer, AMRESCO's Extender™ Taq polymerase blend, dNTPs and electrophoresis loading buffer containing one tracking dye. Once amplification is complete, an aliquot of the PCR reaction can be directly loaded on an agarose gel and migration of PCR products followed by tracking the mobility of the magenta-colored tracking dye (migrating at approximately 10bp on a 1% gel). After electrophoresis, PCR products may be visualized by standard staining methods.

Storage/Stability:

Store at -20°C. Extender PCR-to-Gel Master 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 Extender PCR-to-Gel Master Mix and place on ice.
2. Assemble reactions on ice according to the following table:

Components	Volume (50 µL Reaction)	Final Concentration
Extender PCR-to-Gel Master Mix,, 2X	25 µL	1X
25 µM forward primer	0.5–2.0 µL	0.25–1.0 µM
25 µM reverse 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. DNA bands can be stained and visualized with standard staining methods.

Colony Screening

1. Thaw primers and Extender PCR-to-Gel 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
Extender PCR-to-Gel Master Mix,, 2X	25 µL	1X
25 µM forward primer	0.5–2.0 µL	0.25–1.0 µM
25 µM reverse primer	0.5–2.0 µL	0.25–1.0 µM
Nuclease-Free Water	As needed	–

3. Pick and suspend a colony in the PCR reaction.
4. Remove 5 µL from the PCR reaction and place in a well of a 96-well plate containing 200 µL of LB and antibiotic. Alternatively, the aliquot can be spotted onto a gridded agar 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 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. DNA bands can be stained and visualized with standard staining methods.

Related Products

Code	Product
E476	Water, Sterile, Nuclease-Free
EZ-Vision non-mutagenic, non-toxic fluorescent stain	
N472	EZ-Vision™ One DNA Dye as Loading Buffer
N650	EZ-Vision™ Two DNA Dye as Loading Buffer
N313	EZ-Vision™ Three DNA Dye as Loading Buffer
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

Corporate Headquarters
AMRESCO Inc.
6681 Cochran Road
Solon, OH 44139 USA
Tel: (440) 349-1199
Toll free: 1-800-829-2805 (US & Canada)
Fax: (440) 349-2441
e-mail: info@amresco-inc.com

To Order (USA & Canada)
www.amresco-inc.com
Toll free: 1-800-829-2805
Toll free fax: 1-800-326-3733
Fax: (440) 349-3255
e-mail: dom-sales@amresco-inc.com

International Orders:
Tel: (440) 349-1199
Fax: (440) 349-2441
e-mail: int-sales@amresco-inc.com

Technical Support
Toll free: 1-800-610-2789 (USA & Canada)
Fax: (440) 349-0235
e-mail: techinquiry@amresco-inc.com