Extender™ PCR-to-Gel Master Mix, 2X

Code	Description	Size
N867-2X1.25ML	**Extender™ PCR-to-Gel Master Mix, 2X**	2 x 1.25 mL tubes
N867-1.25ML	**Extender™ PCR-to-Gel Master Mix, 2X**	1 x 1.25 mL tube
N867-1.25ML-SAMPLE	**Extender™ PCR-to-Gel Master Mix, 2X**	1 x 1.25 mL tube

General Information

VWR Life Science AMRESCO's 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 DNA 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 10 bp on a 1% gel). After electrophoresis, PCR products may be visualized by standard staining methods.

Storage/Stability

Store frozen (-20 to 0°C). Stable through 15 freeze thaw-cycles.

Product Use Limitations

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

Protocol/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, 2X and place on ice.
2. Assemble reactions on ice according to the following table:

Component	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 DNA	0.2 – 10 µL	1 – 50 ng
Nuclease-free water	As needed	-

3. Perform standard PCR amplification, example:

Steps	Time	Temperature (°C)
A	2 min	95
B	30 sec	95
C	30 sec	55-65
D	1 min*	68-72
Repeat Steps B-D 29 times		
E	7 min	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 Maser Mix, 2X 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:

Component	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
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 ensure correct identification of positive colonies, numbering should be consistent between PCR reaction tubes 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 about 8 hours.
6. Perform PCR amplification, example:

Steps	Time	Temperature (°C)
A	5 min	95
B	30 sec	95
C	30 sec	55-65
D	1 min*	68-72
Repeat Steps B-D 29 times		
E	7 min	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.

Directions for Use

For Technical Support

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