Ready PCR Mix, 2X

Code	Description	Size
N806-2X1.25ML	**Ready PCR Mix, 2X**	2 x1.25 mL tubes
N806-1.25ML	**Ready PCR Mix, 2X**	1.25 mL tube
N806-1.25ML-SAMPLE	**Ready PCR Mix, 2X**	1.25 mL tube

General Information

VWR Life Science AMRESCO's Ready PCR Mix is supplied as a 2X mixture of reaction buffer, AMRESCO's Extender™ DNA 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 10 bp on a 1% agarose gel) to monitor the DNA migration. After electrophoreses the PCR products are immediately visualized with standard UV illumination without additional post-run staining or destaining steps.

- Direct-to-gel loading after PCR
- Immediate DNA visualization
- Ideal for direct screening of colonies for plasmid
- Safe to use

Storage/Stability

Store at frozen (0 – -20°C). Ready PCR Mix, 2X is 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 Ready PCR Mix, 2X 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 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 below)

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. PCR products can be visualized with a standard ultraviolet light source. The fluorescent EZ-Vision® DNA dye emits in the blue spectrum for documentation.

Colony Screening

1. Thaw primers and Ready PCR 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:

Components	Volume (50 µL reaction)	Final Concentration
Ready PCR 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 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 of a 96-well plate. **Note:** In order to ensure correct identification of positive colonies, numbering should be consistent between the PCR reaction vessel, and the wells of the plate for colony growth.
5. When a sufficient number of colonies have been selected, place the 96-well plate at 37°C and allow to incubate for about 8 hours.
6. Perform PCR reaction (Example program below):

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.

5. Load and separate PCR products on an agarose gel at 5 – 8 V/cm. PCR products can be visualized with a standard ultraviolet light source. The fluorescent EZ-Vision® DNA dye emits in the blue spectrum for documentation.

Directions for Use

For Technical Support

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ZY0574
Rev. 1 12/2015