Hot Start Taq PCR Master Mixes, 2X

Code	Description	Size
1B1408-SAMPLE	**Hot Start Taq PCR Master Mix, 2x**	1 x 1.25 mL tube (50 reactions)
1B1408-100RXN	**Hot Start Taq PCR Master Mix, 2x**	2 x 1.25 mL tubes (100 reactions)
1B1408-500RXN	**Hot Start Taq PCR Master Mix, 2x**	10 x 1.25 mL tubes (500 reactions)
1B1409-SAMPLE	**Hot Start PCR-To-Gel Taq PCR Master Mix, 2X**	1 x 1.25 mL tube (50 reactions)
1B1409-100RXN	**Hot Start PCR-To-Gel Taq PCR Master Mix, 2X**	2 x 1.25 mL tubes (100 reactions)
1B1409-500RXN	**Hot Start PCR-To-Gel Taq PCR Master Mix, 2X**	10 x 1.25 mL tubes (500 reactions)

General Information

VWR Life Science AMRESCO's Hot Start Taq PCR Master Mixes are 2X concentrated, ready-to-use reaction cocktails for routine PCR amplification of DNA fragments up to 4 kb. They contain all the necessary reaction components, except primers and template. Hot Start PCR-to-Gel Taq Master Mix, 2X has the added benefit of containing tracking dyes that migrate at approximately 4 kb and 50 bp to allow direct loading of PCR product on agarose gels following amplification.

Hot Start Taq PCR Master Mixes simplify reaction assembly, improve assay reproducibility, and reduce the risk of contamination. Taq DNA polymerase in the master mix is inactivated with proprietary monoclonal antibodies that bind the polymerase and keep it inactive prior to the initial PCR denaturation step. Upon heat activation (1 minute at 94ºC), the antibodies denature irreversibly, releasing fully active, unmodified Taq DNA polymerase. This enables specific and efficient primer extension with the convenience of room temperature reaction assembly.

Storage/Stability

Store frozen (0 to -20°C).

Product Use Limitations

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

Protocol/Procedure:

Standards 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 Hot Start Taq PCR Master Mix (1B1408 or 1B1409) and place on ice.
2. Assemble reactions according to the table below.

Components	Volume (50 µL reactions)	Final Concentration
Master Mix, 2X	25 µL	1X
10 µM forward primer	0.5 – 2.5 µL	100 – 500 nM
10 µM reverse primer	0.5 – 2.5 µL	100 – 500 nM
5 ng/µL template DNA	0.2 – 10 µL	1 – 50 ng
Nuclease-free Water	As needed	-

3. Perform standard PCR amplification. An example protocol is listed below, but users should optimize the PCR reaction for the intended application.

Step	Time	Temperature
A) Initial Denaturation/Activation of Hot Start Taq	1:00 – 3:00 min	94ºC
B) Denaturation	0:15 – 0:30 sec	94 ºC
C) Annealing	0:30 sec	55 – 65 ºC
D) Extension	1:00* min	68 – 72 ºC
Repeat Steps B – D 29 times		
E) Final Extension	7:00 min	68 ºC
F) Hold	Infinite	4 ºC

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

4. Separate products on an agarose gel at 5 – 8 V/cm. DNA bands can be stained and visualized with standard staining methods.
    A. When using Hot Start Taq PCR Master Mix, 2X:
        a. Mix the desired sample amount with loading buffer before loading onto agarose gel.
    B. When using Hot Start PCR-to-Gel Taq Master Mix, 2X:
        a. Directly load PCR products onto the gel; the loading buffer and tracking dyes are included in the mix.

Frequently Asked Questions

Question	Answer
Why do I not see any product on my gel?	Residual DNase contamination from DNase-treated template RNA
Why does my sample float out of the well?	Reactions were intended to be loaded into agarose gels in TAE buffer. If the running buffer has a higher density than that of TAE, the sample may not sink into the well. Add a density agent such as Ficoll™ or glycerol before loading the gel.
Why do I see multiple or unexpected product sizes?	1. Contamination of another target DNA 2. Multiple DNA target sequences exist, which may necessitate the need for more specific primers 3. Primer dimers

For Technical Support

Toll Free: 1-800-610-2789 (USA & Canada)
Fax: (440) 349-0235
Email: techinquiry@amresco-inc.com

AMRESCO, LLC
A VWR Company

Corporate Headquarters
28600 Fountain Parkway
Solon, Ohio USA 44139-4300

Tel: 440/349-1199
Fax: 440/349-1182
www.amresco-inc.com

Hot Start Taq PCR Master Mixes, 2X

ZY0575
Rev. 1 12/2015