epicentre®
an Illumina® company

TAQXpedite™ PCR System (FAST end-point)

Cat. Nos. TXP78200 and TXP78001

Connect with Epicentre on our blog (**epicentral.blogspot.com**), Facebook (**facebook.com/EpicentreBio**), and Twitter (**@EpicentreBio**).

1. Introduction

The TAQXpedite™ PCR System (FAST end-point)†+* contains a unique blend of thermostable DNA polymerases that can be used for FAST PCR reactions, a carefully optimized 2X Universal MasterMix and a Difficult/Long MasterMix with all four dNTPs and an optimized $MgCl_2$ concentration. The MasterMix also contains Epicentre's patented PCR Enhancer (with betaine+), which substantially improves the yield, efficiency and specificity of amplification of many target sequences, especially those containing a high GC-content or secondary structure.[1-5] In addition, betaine also may enhance PCR by protecting DNA polymerases from thermal denaturation.[6] The TAQXpedite PCR System's Universal MasterMix is designed for routine applications, while the Difficult/Long MasterMix is designed for use with troublesome or long applications. The two MasterMix system offers a convenient, easy-to-use format for most PCR applications in a FAST PCR format. All reaction components are included in the kit. Simply add your template and primers, mix each tube and place in your thermal cycler.

2. Kit Contents

Cat. #	Quantity

TAQXpedite PCR System (FAST end-point) is available in both 200- and 1000- (standard 25-µl) reaction sizes.

TXP78200	**200 reactions**
TAQXpedite PCR Enzyme Blend at 1 U/µl	200 µl
TAQXpedite Universal 2X MasterMix	2.5 ml
TAQXpedite Difficult/Long 2X MasterMix	2.5 ml
Nuclease-Free Water	2.5 ml

TXP78001	**1000 reactions**
TAQXpedite PCR Enzyme Blend at 1 U/µl	1 ml
TAQXpedite Universal 2X MasterMix	12.5 ml
TAQXpedite Difficult/Long 2X MasterMix	12.5 ml
Nuclease-Free Water 2.5 ml	

3. Product Specifications

Storage: Upon receipt, store only at –20°C in a freezer without a defrost cycle. Once the enzyme blend is added to the tube of MasterMix, store at 4°C for up to three months.

Storage Buffer: The TAQXpedite PCR Enzyme Blend is supplied in a 50% glycerol solution containing 50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 0.5% Tween®20, 0.1 mM EDTA, and 1 mM dithiothreitol.

TAQXpedite Universal 2X MasterMix and TAQXpedite Difficult/Long 2X MasterMix:

100 mM Tris-HCl (pH 8.3,), 100 mM KCl, and 400 µM of each dNTP. PCR Enhancer and $MgCl_2$ are also included at the appropriate concentrations for the respective MasterMixes.

Unit Definition: One unit converts 10 nmol of deoxyribonucleoside triphosphates into acid-insoluble material in 30 minutes at 70°C using standard assay conditions.

4. Related Products

- MMLV Reverse Transcriptase 1st-Strand cDNA Synthesis Kit
- MMLV High Performance Reverse Transcriptase
- Baseline-ZERO™ DNase
- RNase-Free DNase I
- MasterAmp™ Real-Time RT-PCR Kit
- MasterAmp™ RT-PCR Kits
- MasterPure™ Nucleic Acid Purification Kits
- FailSafe™ PCR System

Activity Assay: The activity assay is performed in a 50-µl reaction containing 25 mM TAPS (pH 9.3), 50 mM KCl, 2.0 mM $MgCl_2$, 8.5 µg of activated calf thymus DNA, 0.2 mM of each dNTP, and 0.02-0.1 unit of enzyme.

Quality Control: The TAQXpedite PCR System is function-tested to amplify a 539-bp lambda phage sequence in 20 minutes as well as an 80% GC-rich human Fragile X gene fragment in triplicate PCR reactions in 44 minutes.

Contaminating Activity Assays: All components of the TAQXpedite PCR System are free of detectable nonspecific DNase and RNase activities as judged by agarose gel electrophoresis following over-digestion assays.

†,+,* *See page 7 for patent and licensing information.*

5. General Considerations

1. **Template:** DNA prepared using standard isolation techniques is a suitable substrate for amplification. Nevertheless, numerous compounds inhibit amplification including ionic detergents, some gel loading dyes, phenol, and hemin. When purifying templates from agarose gels, minimize exposure to UV irradiation to prevent formation of pyrimidine dimers. Assembly of reactions in a clean area or using positive displacement pipettors with aerosol-barrier tips will minimize the risk of contamination from extraneous DNA templates. We recommend the following amount of template to be included in each end-point PCR reaction, though this may vary depending on the source and quality of the template.[7,8]

• Plasmid DNA:	10 pg	–	1 ng	
• *E. coli* genomic DNA:	50 pg	–	100 ng	
• Human genomic DNA:	5 ng	–	100 ng	
• cDNA:	10 pg	–	10 ng	(≤10% of the reverse transcription reaction volume)

2. **Primer Design:** Primers typically are 15-30 bases in length and contain approximately 50% G+C residues; the annealing temperatures of primer pairs should be nearly identical. Care must be taken to design primers that do not form hairpin loop structures or are self-complementary. The 5′ end of a primer may contain bases that are not complementary with the template; however, the 3′ end of the primer must be complementary with the template. A 2-step cycling program with primers having a T_m closer to 70°C is recommended.

3. **Reaction Components:** The ratio of primer to template is important for controlling the specificity and efficiency of amplification; an excess of primer ensures that the denatured template molecules bind to the primers instead of binding to the complementary DNA strand.[9] Nevertheless, too much primer may lead to the formation of nonspecific products or primer dimers. The recommended primer concentration is 0.2 – 1.0 µM.
4. **Cycling Parameters:** Many parameters influence both the specificity and efficiency of amplification including the temperature and duration of denaturation, annealing and elongation, ramp speed, and total cycle number. Amplification beyond ~10^{12} molecules may also result in the appearance of nonspecific products; if the starting number of template molecules is 10^5, 28-30 cycles will yield 10^{12} molecules.[7] Due to the robustness of the TAQXpedite PCR system, the incubation duration for each step as well as the number of cycles is much reduced. However, variations to the program may be required depending on the amplicon size.

6. Suggested PCR Protocol

Use the following protocol as a guideline for establishing the parameters necessary for amplification of experimental templates. Assemble reactions in a clean area and minimize contamination risk from extraneous DNA templates by using positive displacement pipettors with aerosol-barrier tips.

Assemble the Amplification Reactions:

The volumes listed below are for one 25-µl amplification reaction.

Reaction volumes can be scaled up or down as needed.

1. Thaw and thoroughly mix all of the reagents before dispensing.
   Add 50 µl of the enzyme blend to 625 µl of one of the 2X MasterMixes, and mix. This freshly prepared MasterMix can be stored at 4°C for up to three months.
2. Prepare the reactions. Thaw and thoroughly mix all of the reagents listed below before dispensing. Combine all of the following:

x	µl	nuclease-free water
x	µl	forward primer (0.2-1 µM final concentration)
x	µl	reverse primer (0.2-1 µM final concentration)
x	µl	DNA Template (see recommendations on page 2)
12.5	µl	TAQXpedite PCR MasterMix (made in step 1, above)
25	µl	Total reaction volume

3. Thoroughly mix each tube of reaction mix.

Cycle the Amplification Reactions:

4. Program the thermal cycler following the recommendations provided by the manufacturer.

   A 2-step or 3-step suggested program is outlined below. We recommend a 2-step cycling program for primers with a T_m of 65°C or higher.

Important: *The TAQXpedite PCR Enzyme Blend does not require "hot start" (enzyme reactivation) conditions. Do not include an initial step of prolonged (10-15 minutes) incubation at 95°C. Inclusion of such a step will reduce the subsequent PCR efficiency.*

2-Step Protocol (for primers with a T_m of 65°C or higher)

a) Initially denature the template at 98°C for 30 seconds.

b) Perform 20-35 cycles as required:
Denature at 92-98°C for 1-10 seconds.
Extend the annealed primers at 65-72°C for 10-30 seconds for every kilobasepair of expected product.

3-Step Protocol (for primers with a T_m less than 65°C)

a) Initially denature the template at 98°C for 30 seconds.

b) Perform 20-35 cycles as required:
Denature at 92-98°C for 1-10 seconds.
Anneal at a temperature 2-5°C below the T_m of the primers for 1-10 seconds.
Extend the annealed primers at 65-72°C for 10-30 seconds for every kilobasepair of expected product.

5. Place the tubes in the thermal cycler and begin cycling.

Reverse Transcription Protocol

This is a suggested 2-Step End-Point RT-PCR Protocol for First-strand cDNA Synthesis.

1. Thaw and thoroughly mix all of the reagents listed below before dispensing; place on ice.

   Prepare reaction mixes on ice in the order outlined below:

x µl	nuclease-free water
5 µl	10X RT buffer (i.e. 10X MMLV-RT buffer) (1X final concentration)
5 µl	100 mM dithiothreitol (10 mM final concentration)
x µl	dNTP mix (0.5 mM final concentration of each dNTP)
25 pmol	oligo (dT) primer
– or –	
12.5 pmol	gene-specific primer
1 ng-10 µg	RNA template
5-50 U	Reverse Transcriptase e.g., MMLV-RT)
50 µl	Total reaction volume

2. Incubate at 37°C for 30-60 minutes.
3. Proceed with the end-point PCR as described previously on page 3. Added first-strand cDNA synthesis reaction mix should not constitute more than 10% of the total end-point PCR volume.

7. Troubleshooting Amplification Reactions

Little or no amplification detected

1) Lower annealing temperature. Lower the annealing temperature in 2°C increments.
2) Increase initial template denaturation time. Increase the length of initial template denaturation up to 2 minutes.
3) Redesign primers. Design primers that have higher annealing temperatures and do not form hairpin loops or primer dimers.
4) Optimize primer concentration. Although lower primer concentration can prevent primer-dimer formation, sufficient primers are needed for successful end-point PCR. Increase primer concentration in increments of 50-100 nM.
5) Increase number of cycles. Perform additional cycles in increments of five.
6) Vary DNA polymerase volume. Increasing the amount of DNA polymerase may improve PCR amplification.
7) Check template quality and quality. Check the quality of template DNA by agarose gel electrophoresis or fluorimetry. Organic extraction followed by ethanol precipitation may remove some inhibitors of amplification. We recommend the following amount of template to be included in each end-point PCR (this may vary depending on the source and quality of the template).
   - Plasmid DNA: 10 pg – 1 ng
   - *E. coli* genomic DNA: 50 pg – 100 ng
   - Human genomic DNA: 5 ng – 100 ng
   - cDNA: 10 pg – 10 ng (≤10% of the reverse transcription reaction volume)
8) Increase extension time. Increase the extension time, generally 30 seconds for every kb of product.
9) Decrease RT reaction volume added to the PCR reactions. Too much RT reaction volume added to the end-point PCR may reduce the amplification efficiency. The volume of the RT reaction added to the end-point PCR should not exceed 10% of the total final volume.
10) Avoid contamination of the RT reaction with genomic DNA. We recommend pretreatment of the starting RNA template with Epicentre's Baseline-ZERO™ DNase or RNase-free DNase I. Alternatively, use primers designed at the splice junctions of the target mRNA to avoid amplification of genomic DNA.

Multiple products or a smear detected

1) Decrease concentration of reaction components. Check the concentration of template DNA by agarose gel electrophoresis or fluorimetry. Decrease the amount of enzyme and/or primer added to the reaction.
2) Increase annealing temperature. Increase the annealing temperature in 2°C increments.
3) Redesign primers. Design primers that have higher annealing temperatures and do not form hairpin loops or primer dimers.

4) Check primers for degradation. Check by electrophoresis in a denaturing acrylamide gel.
5) Decrease number of cycles. Decrease number of cycles in increments of five.
6) Avoid contamination of the RT reaction with genomic DNA. We recommend pretreatment of the starting RNA template with Epicentre's Baseline-ZERO DNase or RNase-Free DNase I. Alternatively, use primers designed at the splice junctions of the target mRNA to avoid amplification of genomic DNA.

8. References

1. Schanke, J.T. and Grunenwald, H.L. (1997) *Epicentre Forum* **4** (1), 2.
2. Grunenwald, H.L. and Schanke, J.T. (1997) *Epicentre Forum* **4** (1), 4.
3. Mytelka, D.S. and Chamberlin, M.J. (1996) *Nucl. Acids Res.* **24**, 2774.
4. Henke, W. *et al.*, (1997) *Nucl. Acids Res.* **25**, 3957.
5. Weissensteiner, T. and Lanchbury, J.S. (1996) *BioTechniques* **21**, 1102.
6. Santoro, M.M. *et al.*, (1992) *Biochemistry* **31**, 5278.
7. Roux, K.H. (1995) in: *PCR Primer*, Dieffenbach, C.W. and Dveksler, G.S. (eds.), CSH Press, New York, 53.
8. Kolmodin, L.A. and Williams, J.F. (1997) in: *PCR Cloning Protocols*, White, B.A. (ed.), Humana Press, Totowa, 6.
9. Cha, R.S. and Thilly, W.G. (1995) in: *PCR Primer, Dieffenbach*, C.W. and Dveksler, G.S. (eds.), CSH Press, New York, 37.

Visit our technical blog: **epicentral.blogspot.com**