Hot Start PCR Application

The Taq antibody is used to bind the Taq polymerase and prevents nonspecific amplification due to mispriming and/or formation of primer dimmers during PCR reaction assembly. The antibody is denatured in the initial PCR DNA-denaturation step, releasing the polymerase and allowing DNA synthesis to proceed.

Monoclonal antibody based hot-start PCR has been well recommended and widely used for its excellent performance. Other hot-start methods include chemically modified Taq DNA polymerase- this Taq needs to be activated by incubating at 95 degrees which usually causes depurination and scission of DNA due to the heating-up process.

Titration Test of eENZYME Taq Monoclonal Antibody:

1. Pick up one or several primer set(s) that normally give you high background primer-dimer or non-specific amplification when using Taq polymerase alone, and set up the PCR reaction according to the table at **room temperature.**
2. Prepare antibody and Taq mix as indicated in the following table. Incubate at room temperature for about 10-20 min or 4 ^{o}C overnight.

Ratio (Antibody/Taq)	2:1	1:1	1:2	1:4	1:8	1:10
Anti-Taq (4 µg/µl)	1 µl	1 µl	1 µl	1 µl	1 µl	1 µl
Taq (5 U/µl)	1 µl	2 µl	4 µl	8 µl	16 µl	20 µl

3. Prepare 2 x PCR Master Mix with primers, DNA template added. Make duplicate of each reaction.
4. Set up PCR reaction as indicated in the following table.

Reaction	2 x Master Mix (µl)	Taq Polymerase (mixed w/o anti-Taq)	ddH_2O (µl)	Ratio (Antibody/Taq)
Control	12.5	0.5U	add up to 25 µl	-
1	12.5	0.5U	add up to 25 µl	2:1
2	12.5	0.5U	add up to 25 µl	1:1
3	12.5	0.5U	add up to 25 µl	1:2
4	12.5	0.5U	add up to 25 µl	1:4
5	12.5	0.5U	add up to 25 µl	1:8
6	12.5	0.5U	add up to 25 µl	1:10

5. Place PCR tubes into the real-time thermal cyler and then start the PCR reaction.
6. Analyze the results.

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Real-Time PCR Amplification Protected by Taq Antibodies:

The following data represents end-point high resolution melting analysis of a PCR product created by a primer set which is highly sensitive to sufficient hot-start protection (i.e. antibody function, concentration, conjugation ratio, etc.). The product melt is represented by derivative peaks. A "clean" product melt (Figure 2) shows 2 distinct peaks at ~84 and ~89 degrees. Undesired products created as a result of less-than-adequate hot-start protection are observed as peaks of variable amplitude at ~67 and ~76 with a broad shoulder melt in the range of 79-83 degrees.
In summary, it appears that 40% more antibody is required when using competitor's anti-Taq compared to eEnzyme Anti-Taq (Cat# MA-029-0250). Addition of 40% more antibody of competitor's anti-taq into our mastermix restores hot-start protection in this very sensitive assay.

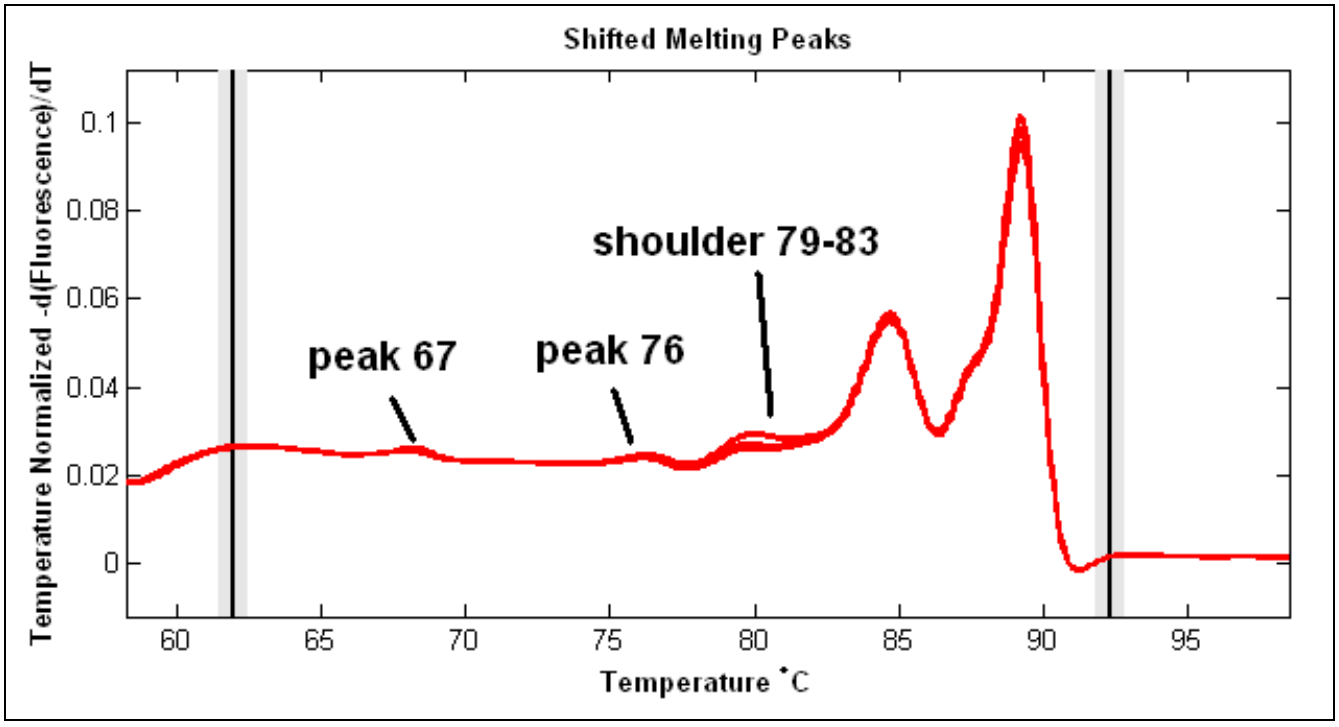

Figure 1. PCR Mastermix without anti-taq antibody. Multiple non-specific amplitudes observed (peak 67, pear 76, and shoulder 79-83).

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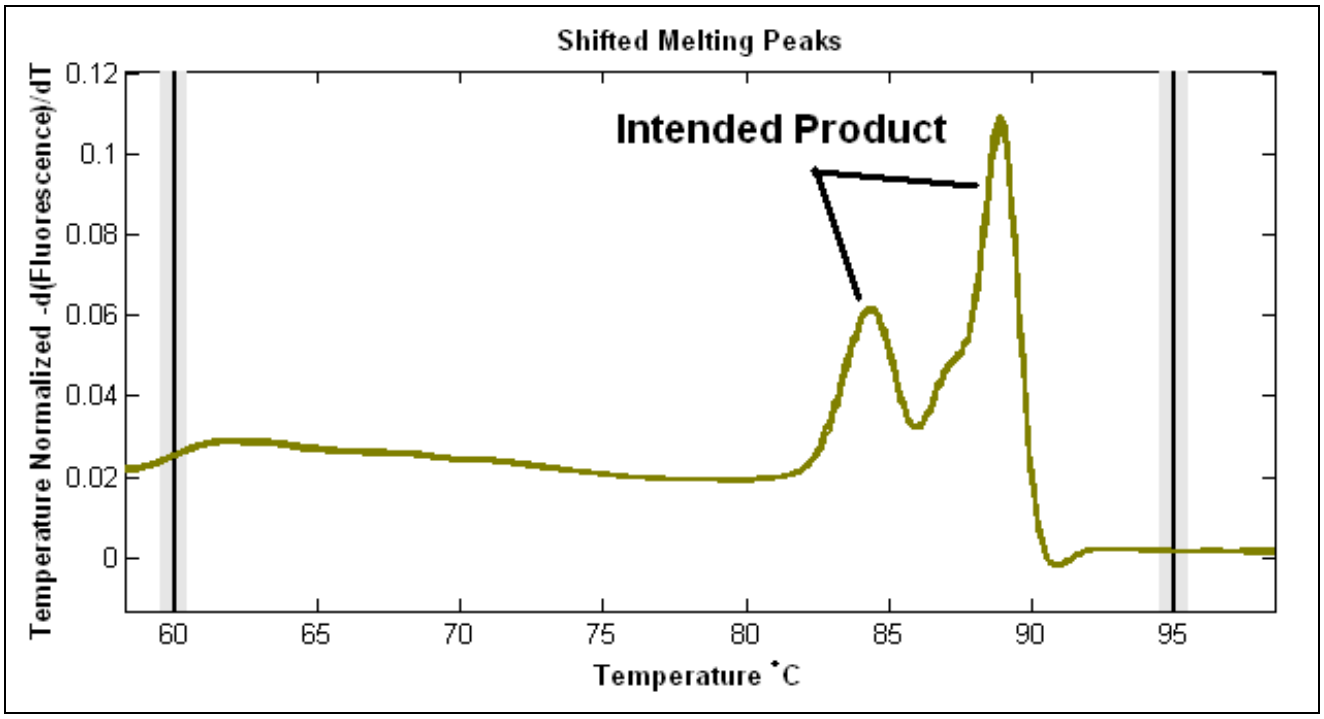

Figure 2. PCR Mastermix (lot 908309) made with eEnzyme anti-Taq (MA-029-0250). A "clean" product melt shows 2 distinct peaks at ~84 and ~89 degrees.

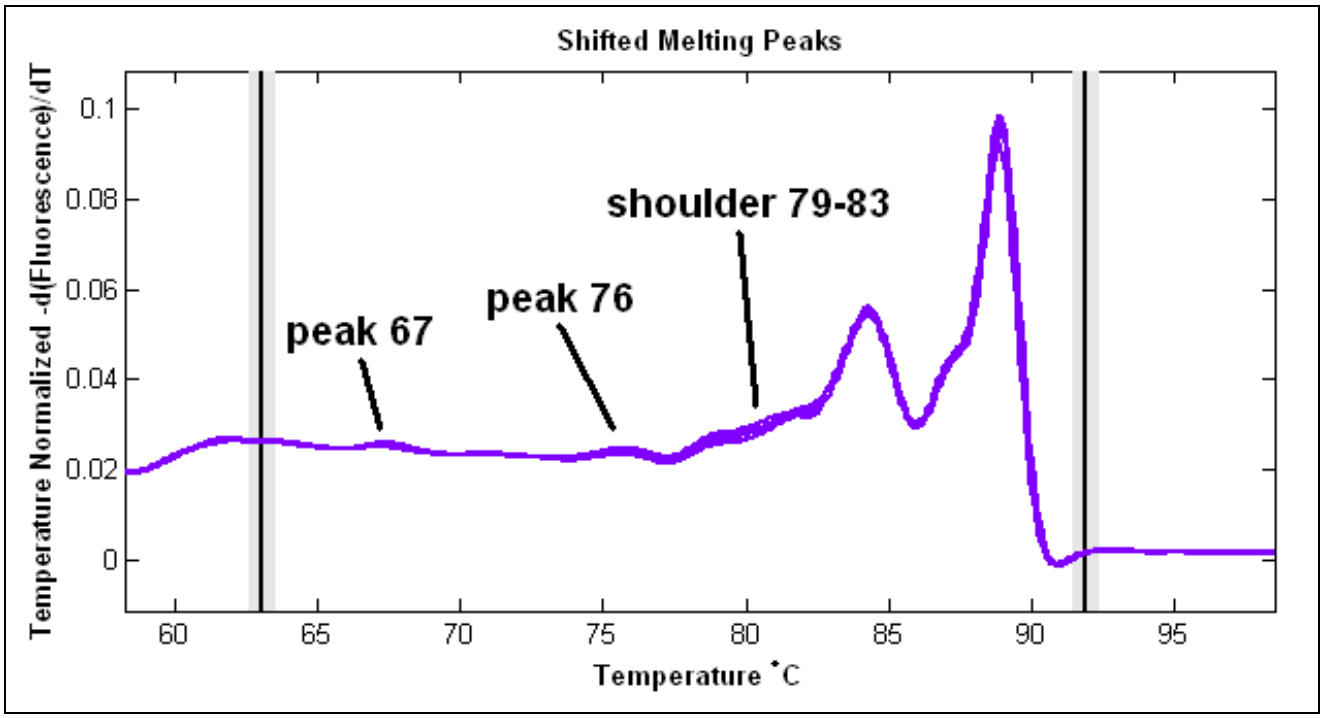

Figure 3. PCR Mastermix (lot 986109) made with competitor's anti-Taq. Undesired products observed as peaks of variable amplitude at ~67 and ~76 with a broad shoulder melt in the range of 79-83 degrees.

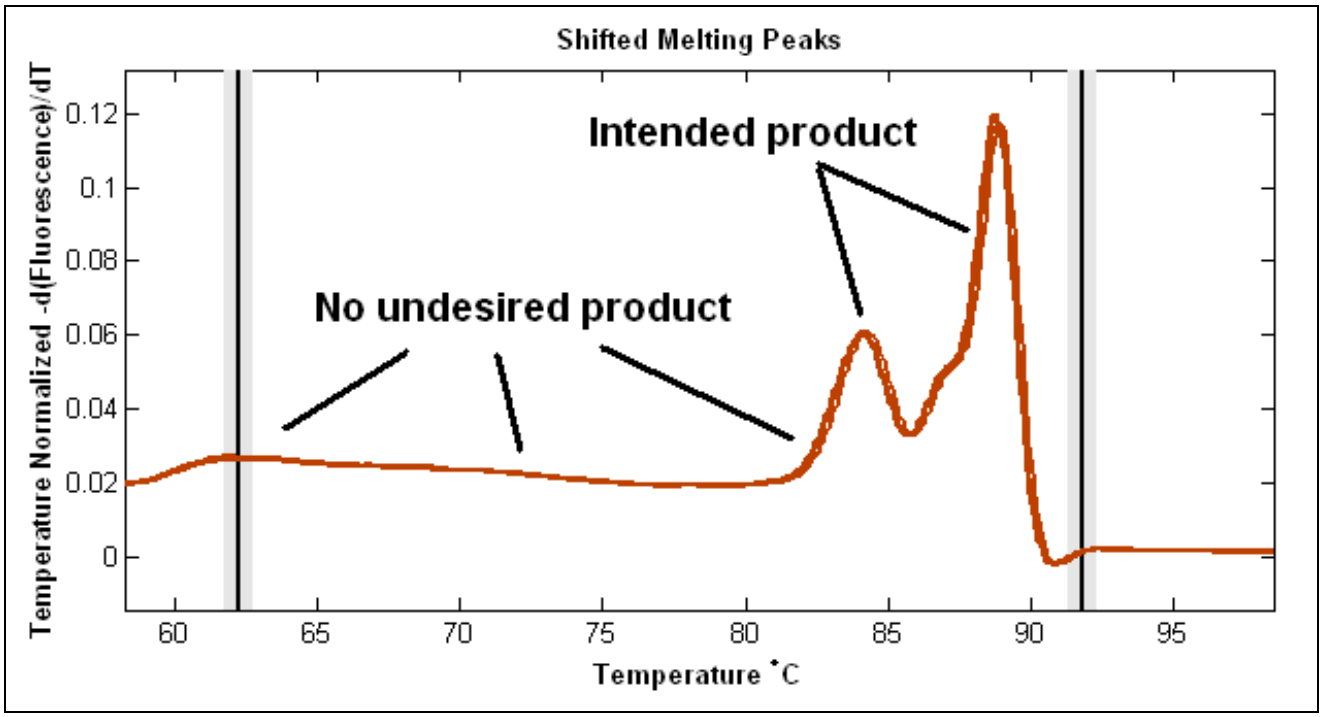

Figure 4. PCR Mastermix (lot 995009) made with competitor's anti-taq, same as PCR mastermix from figure 2 but with 40% more antibody added. Undesired products disappeared.

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