



This tool calculates the T<sub>m</sub> of primers and estimates an appropriate annealing temperature when using different DNA polymerases. **How to use this calculator**

Quickly find the right annealing temperature for **Platinum SuperFi DNA polymerase** (also works for **SuperScript IV One-Step RT-PCR Kit**), **Phusion** and **Phire** DNA polymerases.

**Important note:** If the PCR primer contains desired mismatches, e.g., for creating a mutation or a restriction site, make sure to calculate the T<sub>m</sub> only for the correctly matched sequence

The T<sub>m</sub> calculator is **not required** for **Platinum II Taq DNA Polymerase**, **Platinum SuperFi II DNA Polymerase**, and **Platinum Direct PCR Universal Master Mix**, and **Phusion Plus DNA Polymerase** due to their buffers specially formulated for a universal annealing temperature of 60°C for primers.

1. Select your DNA polymerase

- ☐ Platinum SuperFi DNA polymerase
- (Also select this option if using the SuperScript IV One-Step RT-PCR Kit)
- ☒ Phusion or Phire DNA polymerase
- ☐ DreamTaq DNA polymerase or other Taq-based DNA polymerase

2. Select input method

- ☐ Single pair
- ☒ Batch

3. Paste your sequences

p94 AGTGGGATTCCCTACTCG ; p194 CTCCCGCTCATAGCTTCC  
p95 CCTCCGTTAGATAGCCCT ; p195 TACGGTCTAGAAAAGTCT  
p96 TACTAGTCTCGACCAGTA ; p196 GGAAATTAATTGCTAACT  
p97 AGGCCAATAAGCCTTAAT ; p197 GTACAAGGCAAACCGGTG  
p98 ATGCCGCGGTCCTGAACC ; p198 TGGACCCCTGTCTCACGT  
p99 TTGCACACCCTTGAGGCC ; p199 ACAACTAAGACTCTAGGA  
p100 GTGAGCTGACTTTTCAGT ; p200 TAAACCATAATCACGACT

Clear

4. PCR conditions

Primer conc. 0.5 μM

Results

Export table data into Excel

ID Sequence #1	Molecular weight	Extinction coefficient	Tm °C	ID Sequence #2	Molecular weight	Extinction coefficient	Tm °C	Annealing Temperature °C
TTCTCTTTGGATG	5431.6	159500.0	56.2	p1 AATTAATTATGTATAA	5495.7	188700.0	42.3	42.3
			01 C					

ifference of more than 5°C or greater is not recommended.

ID Sequence #1				ID Sequence #2			
#1	Molecular weight g/mol	Extinction coefficient l/(mol·cm)	Tm °C	#2	Molecular weight g/mol	Extinction coefficient l/(mol·cm)	Tm °C
p2 ATTCGTAGATGTCCAG				p1 ACTAGGGGTATTTCCA			
0 AA	5522.7	185500.0	55.6	20 AC	5498.7	176700.0	56.3
p2 TCATGGGCTTCTCACG				p1 GATACTTTCGAGCATG			
1 GTG	5481.6	161700.0	62.4	21 CC	5474.6	169400.0	58.7
p2 CGCGTGTGCATGGCCG				p1 TGGCATACTGATACCC			
2 GCG	5532.6	161800.0	71.7	22 TT	5449.6	166900.0	58.0
Tm difference of more than 5°C or greater is not recommended.							
p2 CCTCCCCATATACCGC				p1 GCAGCAAGTCGTCAC			
3 CT	5315.5	154200.0	63.3	23 GTG	5524.6	174100.0	64.2
p2 GCGGATGCCCTAATC				p1 AACGGGCGATCTCTA			
4 GCT	5475.6	164100.0	65.3	24 CAC	5468.6	172500.0	61.8
p2 ATGGGCAACCCCTAC				p1 CATGAAACTCTAAACA			
5 CCT	5404.6	163100.0	66.0	25 AA	5468.7	187800.0	50.8
Tm difference of more than 5°C or greater is not recommended.							
p2 GTTTCAATTTTGTATTT				p1 CTAAGGGTGGTTCTT			
6 A	5484.7	173000.0	46.5	26 CGC	5521.6	164800.0	60.5
Tm difference of more than 5°C or greater is not recommended.							
p2 CTGTAGTTAGTACGAG				p1 CGGCTTACAGGCACG			
7 GC	5554.7	178300.0	56.6	27 CGT	5500.6	165600.0	68.2
Tm difference of more than 5°C or greater is not recommended.							
p2 GACCCCGTACGGGG				p1 GGGCGCGCAGTCGG			
8 GGTC	5541.6	169300.0	69.2	28 TTAA	5580.7	174600.0	68.3
p2 TTCGGCCTGTTCATGT				p1 CTGCGACCCGCATCC			
9 CT	5447.6	155800.0	61.8	29 AGC	5405.5	158200.0	68.7
Tm difference of more than 5°C or greater is not recommended.							
p3 CCAGGGTTGCCTTGG				p1 GTGCTGTACGAGGGG			
0 CAA	5515.6	166600.0	66.1	30 CCC	5556.6	168200.0	67.7
p3 GCCAGGCTCCGCGTG				p1 TCGTAGTGCATACGC			
1 CCG	5477.6	156900.0	73.8	31 GAA	5523.7	179200.0	61.7
Tm difference of more than 5°C or greater is not recommended.							
p3 TCATTCGACCTTCGTA				p1 AGTGCATCCGGTCAG			
2 GC	5425.6	163200.0	59.5	32 TCG	5515.6	172500.0	64.7
Tm difference of more than 5°C or greater is not recommended.							
p3 CTA CTCCTGTGATCAG				p1 GCAAACGTCTGCTCA			
3 GC	5410.6	159800.0	58.7	33 AGT	5483.6	171200.0	61.2
p3 CCCTCCGGTTTAATCT				p1 ACAACTCCGTATTCTG			
4 AC	5385.6	159600.0	57.0	34 AG	5458.6	173500.0	56.6
p3 CGCAACTGCGATTGC				p1 CTAGCATGTGACGTAA			
5 TTT	5465.6	160100.0	62.5	35 TT	5513.7	176700.0	55.0
Tm difference of more than 5°C or greater is not recommended.							
p3 GCGACATAAGAGCTC				p1 TGTACGCACGTATAGT			
6 AAG	5541.7	185300.0	58.3	36 AC	5498.7	178800.0	56.3
p3 GATAACGGTCCTACTT				p1 GCGTAGTGCTACCGT			
7 CG	5474.6	171300.0	57.0	37 ACT	5490.6	168800.0	61.5
p3 CACTGATTTTCCA				p1 GTTAAGAGACTATCGA			
	5409.6	163400.0	57.4	38 CA	5531.7	188500.0	53.3

ID Sequence #1	Molecular weight g/mol	Extinction coefficient l/(mol·cm)	Tm °C	ID Sequence #2	Molecular weight g/mol	Extinction coefficient l/(mol·cm)	Tm °C	Annealing Temperature °C
#1				#2				
<div> <div>ThermoFisher Scientific</div> <div>Solutions to support your pre-clinical phase</div> <div>Discover now &gt;</div> </div>								
p3 GTGGGTTTTATTCCGG	5591.7	172900.0	57.6	p1 CGGTATGTATTGCTGC	5520.7	170800.0	57.1	57.1
9 GAT				39 TA				
p4 GTGGTCCTCGGATTA	5514.7	175100.0	59.2	p1 CGCCAGAAGCAAAGC	5486.6	178700.0	63.6	59.2
0 ACA				40 TCA				
p4 TACGCCTTCTCAGCA	5459.6	166800.0	62.1	p1 GATAAAGCCTTAACCT	5466.6	180400.0	51.6	51.6
1 AGG				41 TA				
Tm difference of more than 5°C or greater is not recommended.								
p4 CTCCATGGACATCCA	5443.6	173500.0	58.4	p1 AATACAGTCTGCTACT	5457.6	177800.0	52.5	52.5
2 GTA				42 TA				
Tm difference of more than 5°C or greater is not recommended.								
p4 CCAGCTGACTCTAAA	5468.6	169800.0	60.5	p1 GGGCAGGAGGGTGG	5645.7	180700.0	69.1	60.5
3 GCG				43 TCCA				
Tm difference of more than 5°C or greater is not recommended.								
p4 TAATTTTACCCCTATG	5423.6	171200.0	50.4	p1 ATGGGACAATAACGC	5541.7	184700.0	60.9	50.4
4 A				44 AGC				
Tm difference of more than 5°C or greater is not recommended.								
p4 TATCGATTATCTTAAG	5488.7	177200.0	49.9	p1 TGCTACACGTCGCCG	5491.6	163000.0	67.6	49.9
5 G				45 GTG				
Tm difference of more than 5°C or greater is not recommended.								
p4 GACCTAAAGTAGAGA	5556.7	188000.0	53.6	p1 GGTTCAGCCACGTATA	5459.6	170900.0	60.2	53.6
6 CTG				46 CC				
Tm difference of more than 5°C or greater is not recommended.								
p4 CAGTCATACTTCCGAA	5427.6	173200.0	55.6	p1 AGTAGGTGCTTAGAC	5554.7	178800.0	60.2	55.6
7 AC				47 CGT				
p4 ATGCATGGCCGAGGT	5564.7	176800.0	67.9	p1 GTGAATAGGGGGTATT	5634.7	184900.0	57.1	57.1
8 GCA				48 CG				
Tm difference of more than 5°C or greater is not recommended.								
p4 CGAGATGTTTATTCTT	5519.7	175000.0	52.3	p1 GATTGATCCATGAATG	5553.7	180300.0	55.0	52.3
9 GA				49 GT				
p5 CATCGTCTCTGAAGA	5483.6	173500.0	57.9	p1 TAACGTGTGGCGCTAT	5490.6	166900.0	62.4	57.9
0 ACG				50 CC				
p5 TCCTCGGCCCGTGCC	5378.5	145700.0	70.9	p1 GCTAGCAGATGAATAC	5540.7	190000.0	54.7	54.7
1 TCT				51 AA				
Tm difference of more than 5°C or greater is not recommended.								
p5 TTATAGTGAGCCGAG	5579.7	178800.0	61.4	p1 ACCTTCCCGACTAACT	5363.5	165700.0	60.2	60.2
2 GGC				52 CA				
p5 TGATGTTGAGGGCG	5635.7	179200.0	66.2	p1 CCGCCTATCCCTGAC	5404.6	163300.0	62.3	62.3
3 GGA				53 AAG				
p5 TCAACTCTTGACAGC	5458.6	170000.0	59.1	p1 TTTATGTTACAACGAG	5497.7	179400.0	52.6	52.6
4 GAT				54 AC				
Tm difference of more than 5°C or greater is not recommended.								
p5 TGGTGGACCGTACAA	5529.7	173700.0	59.4	p1 GAATGGCTTCCAGAG	5539.7	174400.0	60.5	59.4
5 TTT				55 GTG				
p5 AGATTAGAATGAAGAC	5564.7	197700.0	52.8	p1 CTATGGCAAATAGCTC	5467.6	175700.0	57.1	52.8
6 CA				56 CA				

ID Sequence #1	Molecular weight	Extinction coefficient	Tm °C	ID Sequence #2	Molecular weight	Extinction coefficient	Tm °C	Annealing Temperature
#1	g/mol	l/(mol·cm)		#2	g/mol	l/(mol·cm)		°C
<div> <div>ThermoFisher</div> <div>SCIENTIFIC</div> </div>	p5 TAAGGGGCCCGTCTT	5523.7	177000.0	61.0	p1 GTGTGATCGTCTGCC	5490.6	169100.0	62.6
	7 AAA			57 ACA				61.0
p5 CCTTAGGGCTCCACA	5420.6	160600.0	64.8	p1 GGGACAGACTGCATT	5508.6	176100.0	62.2	62.2
8 CCG				58 CCA				
p5 CTCAGTTCCTCGA	5419.6	165000.0	59.8	p1 AAAGTCAGGATATTCG	5522.7	182200.0	54.6	54.6
9 AAG				59 TG				
Tm difference of more than 5°C or greater is not recommended.								
p6 GGACCTCCCCCTGCA	5380.5	156000.0	66.7	p1 AATGCTATACTTCGCC	5409.6	163900.0	58.7	58.7
0 ACT				60 CT				
Tm difference of more than 5°C or greater is not recommended.								
p6 TACGTTTATGAAAATG	5497.7	177600.0	53.6	p1 ACACAACATTGCTGTT	5466.6	179500.0	55.6	53.6
1 CC				61 AA				
p6 CAGTTTAAGCCTCGG	5475.6	164500.0	63.4	p1 GCAGAACACCGATGC	5468.6	170500.0	63.4	63.4
2 GCC				62 CTT				
p6 ATAGCCCTGTCTAG	5530.6	174000.0	61.0	p1 ATTAGAATGATTCCAA	5496.7	183800.0	49.1	49.1
3 GTG				63 TT				
Tm difference of more than 5°C or greater is not recommended.								
p6 ATTGTATACTGAAGCG	5562.7	186200.0	56.0	p1 ACCTAAGGGAAGGGC	5558.7	178200.0	67.0	56.0
4 GA				64 GCC				
Tm difference of more than 5°C or greater is not recommended.								
p6 GGTGATTGCTTCCTTT	5502.6	159000.0	57.1	p1 CGCCTACTCTGAACG	5450.6	162000.0	60.7	57.1
5 TG				65 TTG				
p6 CCGATTACGTAAATCT	5458.6	171800.0	56.1	p1 GTGTTTTGAGTTGTAT	5606.7	174600.0	54.2	54.2
6 GC				66 GG				
p6 AAGACCTTCAAGGGA	5531.7	186600.0	55.5	p1 ATTCCTCGCTATGTCG	5441.6	160600.0	62.3	55.5
7 TTA				67 GC				
Tm difference of more than 5°C or greater is not recommended.								
p6 ACAATTCAAATAGCAG	5490.7	185600.0	52.8	p1 GAAGTTAACACCGCT	5507.7	179600.0	55.9	52.8
8 TT				68 TAG				
p6 TAACTCATGCGCCGA	5467.6	176400.0	59.3	p1 AAAGGGTTACCCGAT	5548.7	178900.0	62.6	59.3
9 ATA				69 GGC				
p7 GTTTTTATGTCGTTCT	5476.6	166100.0	50.6	p1 AGGAGGAACACTTAT	5562.7	184500.0	55.8	50.6
0 TA				70 GTT				
Tm difference of more than 5°C or greater is not recommended.								
p7 AAAATAACGGCACATA	5533.7	195200.0	53.6	p1 CAACGTAACGCCACA	5430.6	177700.0	60.1	53.6
1 AG				71 AAC				
Tm difference of more than 5°C or greater is not recommended.								
p7 TCAGGCATTGCATCA	5539.7	173800.0	63.0	p1 TCTCGACAGGACTGA	5459.6	168500.0	60.6	60.6
2 GGG				72 TCC				
p7 ACGTGATACACCGGC	5508.6	177900.0	61.5	p1 TACTAAACAGATAACT	5490.7	185100.0	50.1	50.1
3 TAG				73 GT				
Tm difference of more than 5°C or greater is not recommended.								
p7 CTTGGAAGTGC GGCC	5515.6	165200.0	65.3	p1 CTTATGTCTGAGAACG	5489.6	171200.0	55.0	55.0
4 TGA				74 TC				
Tm difference of more than 5°C or greater is not recommended.								

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Tm difference of more than 5°C or greater is not recommended.

p7 AAGATTGCCTATCCG 6 CG	5474.6	169800.0	60.6	p1 GCCGGAAGCCTATAG 76 ATA	5532.7	184100.0	58.2	58.2
p7 TCGTGAGGTTCAACG 7 TAA	5538.7	180500.0	59.0	p1 TTCGGGCACAACCGA 77 GGC	5509.6	169700.0	68.1	59.0
Tm difference of more than 5°C or greater is not recommended.								
p7 GTTAGCGCCCACCGG 8 GCG	5501.6	163900.0	71.2	p1 GACTGGAGAGACAGC 78 TCC	5533.6	177300.0	61.5	61.5
Tm difference of more than 5°C or greater is not recommended.								
p7 GGGACTCAATCATGG 9 TGG	5579.7	177700.0	60.3	p1 TAACGAATTCGTTTC 79 TT	5454.6	167300.0	52.8	52.8
Tm difference of more than 5°C or greater is not recommended.								
p8 TTACCTGATCGTATCG 0 TG	5480.6	169300.0	56.4	p1 GTTCAATTACACCCTT 80 AA	5417.6	174000.0	52.5	52.5
p8 TCAACTCTCTGTGCTT 1 CT	5391.6	152600.0	58.4	p1 TAAGGAGCTGACATC 81 CAC	5492.6	177600.0	58.9	58.4
p8 TGCGAGGAATATTCCA 2 GA	5547.7	184200.0	58.3	p1 AGCTTATATCAGCGCT 82 CG	5474.6	170800.0	59.7	58.3
p8 TACGGAAGGGCACAT 3 CGG	5573.7	180500.0	64.5	p1 ACGAGACGCGCGACA 83 CAC	5487.6	177300.0	67.9	64.5
p8 TACGGTAGATTGTTTA 4 AA	5552.7	185600.0	51.1	p1 TGACTGAATATTGGA 84 CT	5553.7	177700.0	55.6	51.1
p8 TCAGGATAGATGCCC 5 CTT	5474.6	170100.0	60.2	p1 TGTCCATTCAAACCAG 85 GC	5443.6	169400.0	60.8	60.2
p8 CCATTGCATGGGGCC 6 TAG	5515.6	167500.0	63.4	p1 AACCGACCTGCCCTC 86 CTG	5380.5	156900.0	66.9	63.4
p8 GCCTCGTTTATGAAAA 7 GG	5538.7	177200.0	56.6	p1 ATGACGGCCAGCATC 87 GTT	5499.6	172000.0	65.1	56.6
Tm difference of more than 5°C or greater is not recommended.								
p8 TCCCCGGGTCTGGTC 8 ACA	5451.6	162200.0	68.0	p1 TTGTGCAGCGGATCG 88 CTT	5521.6	163400.0	65.6	65.6
p8 TGAACACTTATACGCT 9 GT	5473.6	171900.0	56.5	p1 CGTGCAATCCAGCAA 89 GAT	5492.6	176800.0	61.1	56.5
p9 TTAAGCCAAGTCTCC 0 AA	5451.6	178100.0	56.7	p1 GTGATCGGAATTCCT 90 AG	5514.7	174800.0	57.1	56.7
p9 AGCGCCATTTGGGTG 1 AGG	5595.7	175100.0	65.8	p1 TAGAAGGGTTCGTGG 91 CAG	5619.7	181600.0	61.8	61.8
p9 TAGCTCAATGTGACAG 2 CT	5498.7	173500.0	58.9	p1 TAGCCCGACAACCAA 92 ATG	5461.6	177900.0	60.5	58.9
p9 GGTCTAGGCTAGGAGC 3 TCC	5540.6	171300.0	64.0	p1 AGGTATTGTTTCCAC 93 CG	5465.6	168100.0	59.9	59.9
p9 AGTGGGATCCCTACT 4 CG	5490.6	169300.0	60.7	p1 CTCCCGCTCATAGCTT 94 CC	5346.5	151300.0	62.3	60.7

## Platinum SuperFi II DNA Polymerase