



Tm Calculator

v 1.12.0



Product Group

Q5

Polymerase/Kit

Q5 High-Fidelity DNA Polymerase

Primer Concentration (nM)

500

Primer Pairs

Type/paste sequences below or load primer pairs from file:

Choose File

No file chosen

Interleaved data (one primer per line)

P1fwd; AGCGGATAACAATTTTCACACAGGA; P1rev; GTAAAACGACGGCCAGT
pBeta; AGCGGATAACAATTTTCAC
P3fwd; AGCGGATAAGGGCAATTTTCAC; P3rev; GTAAAACGACGGCCA

5 primers processed. Errors: 1 Warnings: 1

DOWNLOAD FILE

ID	Sequence	Tm	Ta	Notes
P1fwd	AGCGGATAACAATTTTCACACAGGA	66	63	OK
P1rev	GTAAAACGACGGCCAGT	62	63	OK
pBeta	AGCGGATAACAATTTTCAC	57	---	Primer 2 missing. -- Both primers need to be longer than 7 nt

ID	Sequence	Tm	Ta	Notes
P3fwd	AGCGGATAAGGGCAATTCAC	65	60	OK--Tm difference is greater than the recommended limit of 5 °C.
P3rev	GTAAAACGACGGCCA	59	60	OK--Tm difference is greater than the recommended limit of 5 °C.

Use of the Q5 High GC Enhancer often lowers the range of temperatures at which specific amplification can be observed, however the rule used to determine Q5 annealing temperatures ($T_a = T_{m_lower} + 1^{\circ}\text{C}$) typically yields values that will support specific amplification with or without the enhancer.