# $T_m$ Calculator

This tool calculates the $T_m$ 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 $T_m$ only for the correctly matched sequence

The $T_m$ calculator is **not required** for **Platinum II *Taq* DNA Polymerase**, **Platinum SuperFi II DNA Polymerase**, and **Platinum Direct PCR Universal Master Mix** due to their buffers specially formulated for universal annealing at 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

P1fwd AGCGGATAACAATTTCACACAGGA; P1rev GTAAAACGACGGCCAGT
pBeta AGCGGATAACAATTTCAC; xxx GGGGGGGGGGGGGGGGGGGG
P3fwd AGCGGATAAGGGCAATTTCAC; P3rev GTAAAACGACGGCCA

Clear

### 4. PCR conditions

**Primer conc.** 0.5 µM

### Results

Export table data into Excel

| ID #1 | Sequence #1 | Molecular weight g/mol | Extinction coefficient l/(mol˙cm) | Tm °C | ID #2 | Sequence #2 | Molecular weight g/mol | Extinction coefficient l/(mol˙cm) | Tm °C | Annealing Temperature °C |
|---|---|---|---|---|---|---|---|---|---|---|
| P1fw | AGCGGATAACAATTTCACACAGGA | 7378.9 | 248700.0 | 65.7 | P1rev | GTAAAACGACGGCCAGT | 5228.5 | 172500.0 | 60.2 | 60.2 |
| Tm difference of more than 5°C or greater is not recommended. | | | | | | | | | | |
| pBAC et a | AGCGGATAACAATTTCAC | 5491.7 | 181900.0 | 55.9 | xx GGGGGGGGGGGGGGx | GGGGGGG | 6851.4 | 213500.0 | 85.1 | 55.9 |
| Tm difference of more than 5°C or greater is not recommended. | | | | | | | | | | |
| P3fw | AGCGGATAAGGGCAATTTCAC | 6479.3 | 212000.0 | 64.1 | P3rev | GTAAAACGACGGCCA | 4595.1 | 154400.0 | 56.4 | 56.4 |
| Tm difference of more than 5°C or greater is not recommended. | | | | | | | | | | |

**Ready to order primers? ›**

## How to use the $T_m$ calculator

The calculator calculates recommended $T_m$ (melting temperature) of primers and PCR annealing temperature based on the primer pair sequence, primer concentration, and DNA polymerase used in PCR. The calculator also calculates the primer length, percentage of GC content, molecular weight, and extinction coefficient.

The modified Allawi & SantaLucia's thermodynamics method [1] is used for $T_m$ and annealing temperature calculation of reactions with Platinum SuperFi, Phusion and Phire DNA Polymerases. The parameters were adjusted on a set of primers seeking to maximize specificity and retain high yields.

To use this calculator select your DNA polymerase, type in or paste your primer sequences, and provide your final primer concentration. $T_m$ values, annealing temperature, and other data are automatically generated.

If necessary, use a temperature gradient to further optimize and empirically determine the ideal annealing temperature for each template-primer pair combination. The annealing temperature gradient should start with temperature 6–10 °C lower than annealing temperature generated by the calculator and increased up to the extension temperature (**two-step PCR**).


1. **Allawi, H. T., and SantaLucia, J. (1997). Thermodynamics and NMR of internal G-T mismatches in DNA. *Biochemistry*, 36(34), 10581-10594.**


## Additional technical resources

- **Optimizing $T_m$ and primer annealing**
- **Oligos tools and utilities**
- **Molecular biology web tools**
- **Molecular biology resource library**
- **Invitrogen School of Molecular Biology**

## Related products

- **DreamTaq DNA polymerase**
- **Phusion High-Fidelity DNA polymerase**
- **Platinum SuperFi DNA polymerase**

Portugal