

# Melting Temperature

Determining the melting temperature,  $T_m$ , of an oligo is essential for many applications such as PCR, capture assays, mutagenesis, hybridization, and sequencing. The exact  $T_m$  of your DNA can be determined only by empirical means.<sup>1</sup> However, the three theoretical methods described here can approximate the  $T_m$ . Selecting the appropriate equation depends on your application.

## What is $T_m$ ?

The  $T_m$ , or melting temperature, of an oligo is the temperature at which 50% of the oligonucleotide and its perfect complement are in duplex. Typically, annealing or hybridizations are performed at 5-10 °C below the  $T_m$  of a duplex. Failing to calculate the correct  $T_m$  for an oligo could result in inappropriate duplex formation. Primer mismatch, false priming, and background signal problems could result if annealings and hybridizations are performed at temperatures significantly below the oligo  $T_m$ . Using temperatures well above the  $T_m$  of an oligo could result in reduced priming, or no priming or hybridization. The main factors affecting  $T_m$  are salt concentration, DNA concentration, the presence of denaturants (formamide or DMSO), DNA sequence, and length.

## Nearest Neighbor Method

At Sigma-Genosys, we use the nearest neighbor method to determine the  $T_m$  of oligonucleotides. This equation is considered to be one of the more accurate derivations of  $T_m$ . The nearest-neighbor method takes into account the actual sequence of your oligo, whereas the other equations rely on the base composition to calculate  $T_m$ . With the nearest-neighbor method, several oligos with the same length and base composition, but differing sequences, would have a different  $T_m$ . In Table 1, each of the sequences contain 5 As, 5 Cs, 5 Gs, and 5 Ts. Notice the  $T_m$  varies with each sequence despite the base composition being the same.

**Table 1. Varying values of sequences with the same GC content.**

Sequence (5'-3')	$T_m$	MW	LEN	GC
AAAAACCCCGGGGGTTTT	69.7	6103	20	50%
ACGTACGTACGTACGTACGT	57.2	6103	20	50%
GATCGATCGATCGATCGATC	64.5	6103	20	50%
ATATATATATCGCGCGCGCG	66.4	6103	20	50%

The nearest-neighbor method incorporates certain variables such as salt concentration and DNA concentration. Sigma-Genosys uses conditions typically found in PCR applications (50 mM monovalent salt and 0.5  $\mu$ M primer). The nearest-neighbor equation for DNA and RNA-based oligos is:<sup>2</sup>

$$(1) T_m = (1000\Delta H/A + \Delta S + R \ln(C/4)) - 273.15 + 16.6 \log[\text{Na}^+]$$

$\Delta H$  (Kcal/mol) is the sum of the nearest-neighbor enthalpy changes for hybrids.  $A$  is a small, but important constant containing corrections for helix initiation.  $\Delta S$  is the sum of the nearest-neighbor entropy changes.  $R$  is the Gas Constant (1.99 cal  $K^{-1}mol^{-1}$ ), and  $C$  is the concentration of the oligo. The  $\Delta H$  and  $\Delta S$  values for nearest-neighbor interactions of DNA and RNA are shown in Table 2. In many cases this equation gives values that are no more than 5 °C from the empirical value. Please note that this equation includes a factor to adjust for salt concentration.

**Table 2. Thermodynamic parameters for nearest-neighbor melting temperature formula.**

Interaction	DNA		RNA	
	$\Delta H$	$\Delta S$	$\Delta H$	$\Delta S$
AA/TT	-9.1	-24.0	-6.6	-18.4
AT/TA	-8.6	-23.9	-5.7	-15.5
TA/AT	-6.0	-16.9	-8.1	-22.6
CA/GT	-5.8	-12.9	-10.5	-27.8
GT/CA	-6.5	-17.3	-10.2	-26.2
CT/GA	-7.8	-20.8	-7.6	-19.2
GA/CT	-5.6	-13.5	-13.3	-35.5
CG/GC	-11.9	-27.8	-8.0	-19.4
GC/CG	-11.1	-26.7	-14.2	-34.9
GG/CC	-11.0	-26.6	-12.2	-29.7
Initiation	0.0	-10.8	0.0	-10.8



## Other Equations

The simplest equation for  $T_d$  is the Wallace rule<sup>3</sup>:

$$(2) T_d = 2(\#A + \#T) + 4(\#C + \#G)$$

$T_d$  is for membrane-based calculations where A, G, C, and T are the number of occurrences of each nucleotide in the oligo. This equation was developed for short DNA oligos of 14-20 bases hybridizing to membrane-bound DNA targets in 0.9 M NaCl.

The  $T_m$  for GATC GATC GATC GATC GATC is  $2(5 + 5) + 4(5 + 5) = 60$  °C. If both target and probe are free in solution, the  $T_m$  is approximately 7-8 °C lower than when the target is immobilized on a membrane.

Another familiar equation<sup>4,5</sup> for DNA which is generally used for oligos longer than 50 bases at pH 5.0 to 9.0 is the % GC method:

$$(3) T_m = 81.5 + 16.6 \log[Na^+] + 41(X_G + X_C) - 500/L - 0.62F$$

where  $[Na^+]$  is the molar concentration of monovalent cations (in this case  $Na^+$ ),  $X_G$  and  $X_C$  are the mole fractions of G and C in the oligo, L is the length of the shortest strand in the duplex, and F is the percentage of formamide in the hybridization solution.

In a hybridization application, the salt concentration is a variable, but an essential component for the kinetics of the DNA strands. Formamide tends to denature the helix and bonds to the single strands, thereby lowering the  $T_m$ . When salt and formamide are added to a hybridization reaction, the hybridization temperature changes. The % GC method adjusts for salt and formamide making it a useful equation (although the equation is undefined when  $[Na^+] = 0$ ).

## Conclusion

As with any theoretical approach, the results of these equations should be used with caution. Some experiments may involve reagents or conditions for which the results of these equations are not suitable. In these cases, only an empirical approach may provide the most satisfactory answer.

Counter-ion identity, modifications (biotin, fluorescent dyes, etc.), solvation effects, and impurities may also affect the  $T_m$ . Oligo modifications tend to decrease the  $T_m$ . The magnitude of the decrease depends on the location of the modification (internal being more destabilizing than terminal), and on the overall  $T_m$  of the unmodified sequence. In these cases, theoretical equations are inaccurate, but still provide a useful estimate.

## Glossary

**$T_m$**  — The melting temperature is the temperature (in °C) at which 50% of the oligo and its perfect complement are in duplex.

**$T_d$**  — The dissociation temperature is the temperature (in °C) at which 50% of an oligo and its perfect filter-bound complement are in duplex at a particular salt concentration, and total strand concentration.

**$\Delta H$**  — Enthalpy change is calculated by subtracting the bond energies of the products from the bond energies of the reactants.

**$\Delta S$**  — Entropy change is the tendency for randomness in a system. Typically, conditions in nature have a tendency toward low enthalpy and high entropy.

## References

1. The common way to determine the actual melting point is to use a thermostatted cell in a UV spectrophotometer. If temperature is plotted vs. absorbance, an S-shaped curve with two plateaus will be observed. The absorbance reading halfway between the plateaus corresponds to  $T_m$ .
2. For DNA see: Breslauer, K.J.; Frank, R.; Blocker, H.; Marky, L.A. *Proc. Natl. Acad. Sci. USA* **83**, 3746-3750 (1986). For RNA see: Freier, S.M.; Kierzek, R.; Jaeger, J.A.; Sugimoto, N.; Caruthers, M.H.; Neilson, T.; Turner, D.H. *Proc. Natl. Acad. Sci.* **83**, 9373-9377 (1986).
3. Wallace, R.B.; Shaffer, J.; Murphy, R.F.; Bonner, J.; Hirose, T.; Itakura, K. *Nucleic Acids Res.* **6**, 3543 (1979).
4. Howley, P.M.; Israel, M.F.; Law, M.F.; Martin, M.A. *J. Biol. Chem.* **254**, 4876 (1979).
5. The equations for RNA or RNA-DNA hybrids are:  $T_m = 79.8 + 18.5 \log[Na^+] + 58.4 (X_G + X_C) + 11.8 (X_G + X_C)^2 - 820/L - 0.35F$ , and for DNA-RNA hybrids:  $T_m = 79.8 + 18.5 \log[Na^+] + 58.4 (X_G + X_C) + 11.8 (X_G + X_C)^2 - 820/L - 0.50F$ . These equations are derived for oligo-immobilized target hybrids. In general, one can say that the stability for different hybrids is RNA-RNA > RNA-DNA > DNA-DNA.

For an interactive  $T_m$  demonstration, visit our Web site at [sigma-genosys.com](http://sigma-genosys.com). Provide your sequence of interest, and the  $T_m$  will automatically be calculated for you.



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World Headquarters • 3050 Spruce St., St. Louis, MO 63103 • (314) 771-5765

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