Equations for Calculating Tm

SYSTEM	EQUATION[a]	REFERENCE
DNA-DNA hybrid[a,b]	Tm= 81.5°C + 16.6(logM) + 0.41(%GC) -0.61(%form) - 500/L	1
DNA-RNA hybrid[a,b]	Tm= 79.8°C + 18.5(logM) + 0.58(%GC) + 11.8(%GC)^2 -0.50(%form) -820/L	2
RNA-RNA hybrid[a,b]	Tm= 79.8°C + 18.5(logM) + 0.58(%GC) + 11.8(%GC)^2 -0.35(%form) -820/L	3
Oligonucleotide probes	Tm= 2(# AT bp) + 4(# GC bp)	4

a. M: molarity of monovalent cations (Na^+ concentration); % GC: % G and C nucleotides in the DNA; % form: percentage of formamide in the hybridization solution; L: length of the duplex in base pairs; N: chain length.

b. These equations hold for Na^+ concentrations between 0.01 and 0.40 M and % GC values of 30-75%.

REFERENCES

1. Maniatis, T., et al (1982) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press: New York.
2. Casey, J. and Davidson, N. (1977) *Nucleic Acids Res*., 4: 1539.
3. Bodkin, D.K. and Knudson, D.L. (1985) *J. Virol. Methods*, 10: 45.
4. Wallace, R.B., et al. (1979) *Nucleic Acids Res*. 6: 3545.

Determination of Nucleic Acid Concentration

1. Measure DNA or RNA concentration using the A_{260} value. Please note that this measurement does not discriminate between RNA and DNA.
2. Water is recommended as the solvent for measuring DNA or RNA concentration.
3. Use the same solvent to zero the spectrophometer before measuring the sample.
4. Ensure that the cuvettes are RNase-free for measuring RNA samples.
5. Use the following conversion to determine the concentration of nucleic acid in your sample:

A_{260} value of 1 = 50 µg/ml dsDNA
A_{260} value of 1 = 37 µg/ml ssDNA
A_{260} value of 1 = 40 µg/ml ssRNA

Example: Measuring DNA concentration

Volume of DNA = 50 µl

Dilution: 10 µl DNA sample + 490 µl distilled H_2O (1/50 dilution)

A_{260} of diluted sample (1 cm length) = 0.75

DNA Concentration = 50 µg/ml x A_{260} x dilution factor
= 50 µg/ml x 0.75 x 50
= 1875 µg/ml

Total Amount of DNA = concentration x volume of sample in ml
= 1875 µg/ml x 0.050 ml
= 93.75 µg

SI Units

Prefix	Factor	Abbreviation
atto	10^{-18}	a
femto	10^{-15}	f
pico	10^{-12}	p
nano	10^{-9}	n
micro	10^{-6}	µ
milli	10^{-3}	m
centi	10^{-2}	c
deci	10^{-1}	d
deca	10^{1}	da
hecto	10^{2}	h
kilo	10^{3}	k
myria	10^{4}	my
mega	10^{6}	M
giga	10^{9}	G
tera	10^{12}	T
peta	10^{15}	P
exa	10^{18}	E

Determination of Nucleic Acid Purity

1. Measure the nucleic acid purity using A_{260}/A_{280} ratio.
2. Use low salt buffers as they provide a more accurate measurement. Purity is influenced by pH and lower pH solutions lower the A_{260}/A_{280} ratio and reduce the sensitivity to protein contamination[1].
3. Pure DNA has an A_{260}/A_{280} ratio of 1.8-2.0 in 10 mM Tris, pH 8.5.
4. Pure RNA has an A_{260}/A_{280} ratio of 1.9-2.1 in 10 mM Tris, pH 7.5.

Detecting Contamination

1. Absorbance at 230 nm and 270 nm indicates the presence of phenol or urea[2, 3].
2. Absorbance at 280 nm (hence, a low A_{260}/A_{280} ratio) indicates the presence of protein.
3. Absorbance at 325 nm indicates contamination by particulates and/or dirty cuvettes.

REFERENCES

1. Wilfinger, W. W., Mackey, M.A., and Chomczynski, P. (1997) *BioTechniques* 22: 474
2. *Current Protocols in Molecular Biology*, Ausubel, F. M. et al. Eds
3. Stulnig, T.M. and Amberger, A. (1994) *BioTechniques* 16: 403