

Guidelines for PNA oligomers

Handling and storage of PNA oligomers

1. Spin down the tube and dissolve in water to make a stock of 20~100 μ M stock. For example, if you dissolve 500 μ l of water for 50 nmole PNA, the stock will be 100 μ M. PNA is most stable in water.
2. For purine-rich (>60%), high G content (>35%), or long PNA oligomers (>25 bp), solubility might be reduced in an aqueous solution. If PNA is not completely dissolved in water, add organic solvents such as DMSO or DMF to up to 10% final concentration.
3. Store in aliquots at 4 $^{\circ}$ C for the short term and -20 or -70 $^{\circ}$ C for the long term.
4. For fluorescently labeled probes, protect from light.
5. When taking out the frozen stock, heat the solution at 55 $^{\circ}$ C for 5 min and vortex well before using it.
6. PNA is a very stable molecule and should be intact for over 3 years if properly stored.

General rules for PNA oligomers

1. PNA can form a duplex with target nucleic acid in either orientation but antiparallel is strongly preferred.
2. PNA has higher affinity and specificity. T_m of PNA is about 1 $^{\circ}$ C per base pair higher than DNA. There is about a 15 $^{\circ}$ C T_m difference for single base mismatch for PNA while it is about 10 $^{\circ}$ C for DNA.
3. Purine-rich PNA oligomers have reduced solubility, and tend to aggregate. It is recommended that the purine content of an oligomer is less than 60% and no more than 6 stretches of purine (especially G base) in an oligomer.
4. Due to the neutral backbone of PNA, the binding of PNA to nucleic acid is less dependent on ionic strength and pH. PNA binding to DNA or RNA is not affected significantly from 10 mM to 1 M of NaCl concentration. However, when a salt concentration is lower than 10mM, non-specific binding can occur.
5. Please refer PNA Tool on our website for design guidelines and T_m calculation.
http://www.pnabio.com/support/PNA_Tool.htm

PNA Quantification

1. Calculate the molar extinction coefficient (ϵ_{260}) of a PNA oligomer.
$$\epsilon_{260}(\text{PNA oligomer}) = \sum \epsilon_i \times n_i$$

(i = A, G, C and T, ϵ_i = molar extinction coefficient of base, n_i = number of base)

$$\epsilon_{260}(\text{A}) = 13.7 \text{ ml}/(\mu\text{mole} \times \text{cm}), \epsilon_{260}(\text{C}) = 6.6 \text{ ml}/(\mu\text{mole} \times \text{cm})$$
$$\epsilon_{260}(\text{G}) = 11.7 \text{ ml}/(\mu\text{mole} \times \text{cm}), \epsilon_{260}(\text{T}) = 8.8 \text{ ml}/(\mu\text{mole} \times \text{cm})$$
2. Make a stock solution by dissolving the PNA oligomer in the appropriate volume (V_A) of water/or buffer.
3. Take an appropriate volume (V_B) of the PNA solution and dilute it as needed.
4. Measure the absorbance of the diluted PNA solution at 260 nm using a spectrophotometer.
5. Determine the nanomolar amount of the total PNA oligomer using the formula below.
$$\text{PNA concentration (mM)} = (\text{A}_{260} / \epsilon_{260}) / \text{path length (cm)} \times \text{dilution factor (} V_A / V_B \text{)}$$