Frequently Asked Questions about PNA

1. Why does PNA cost more than DNA?

PNAs are priced more like synthetic peptides than synthetic DNA. PNAs are chemically synthesized by the same methods used to make peptides which can cost thousands of dollars for a custom synthesis. As the use of PNA grows the prices for both PNA custom synthesis and PNA synthesis reagents will decrease due to economies of scale.

2. In what buffers is PNA soluble?

Water, neutral buffers, concentrated salt solutions, DMF or ACN. We recommend at least 10 millimolar buffer or salt so the PNA will have a higher affinity for the solution versus the container. PNAs are generally purified if they are ten or more bases long. Purification is done on reverse phase C-18 columns with water/acetonitrile each with a small amount (0.1%) of TFA. The dried down fractions are readily soluble in 0.1% TFA which serves as an excellent solvent for stock solutions.

3. How much do you get when you order a custom PNA?

For a standard 1 micromole scale custom PNA of about 18 mers there is about 50-70 O.D. at 260 nm (~26 ug/O.D.) of crude product and after purification you would receive about 5-20 O.D. of purified product. For modified PNAs that contain complex peptides or nonstandard labels, yields of final purified product may be less.

4. How long can you make a PNA?

Although we have made longer PNAs, an 18 mer is usually our recommended maximum length. PNA oligomers 12-15 bases long have worked well experimentally for a number of applications. By 18 bases you get a maximal increase in Tm and specificity. By making them longer problems with solubility, aggregation will arise.

5. Why can't I use PNA as a primer for my PCR?

PNAs do not have a 3'-hydroxyl end and are not a recognized substrate for any form of DNA Polymerase or any other enzyme (yet). Since PNA has a backbone like a peptide, it has an Amino (5' analog) end and a Carboxy (3' analog) end. A PNA of sufficient length will inhibit PCR either by competition for the primer site or by blocking DNA

Polyermase (TAQ, vent, Klenow etc.) Since PNAs bind tighter and with greater specificity, they are much less tolerant of a single base mismatch than the DNA analog. They will selectively suppress the amplification of a perfect match and not inhibit the amplification of a sequence that differs by only one base. This technique, known as PCR Clamping, imparts the ability to resolve all single base differences between sequences you wish to amplify or detect.

6. How can I determine the concentration of my PNA?

Standard spectophotometric techniques apply. You can calculate extension coefficients for PNA by using the same formulas used for DNA, or you may use the average value of about 26 micrograms of single stranded PNA per O.D. at 260 nanometers. Additional information is available in our *How to Use Your PNA* handout.

7. How can I determine the extinction coefficient of my PNA?

We use 26 micrograms per O.D. at 260 nm. You may use the standard calculations for DNA extension coefficients.

8. What are the molecular weights of the PNA units in the final PNA or how do I calculate the formula weight of a PNA?

Molecular weights of Expedite PNA monomers are:

Fmoc-T-OH	MW	506.52
Fmoc-A (Bhoc)-OH	MW	725.77
Fmoc-C (Bhoc)-OH	MW	701.74
Fmoc-G (Bhoc)-OH	MW	741.77

Please refer to additional information contained in our *How to use Your PNA* handout.

9. Can I get a mix of PNA and DNA (PNA-DNA Chimera)?

Although it is possible to make a chimeric molecule that is part PNA part DNA, we do not offer this as a custom synthesis product at this time.

10. How do you know which end of the PNA is the 5' end?

The amino terminal is analogous to the 5' end of DNA.

11. At what concentration does PNA precipitate?

PNA solubility is sequence and size dependent. Also, the presence of Amino acids, label(s) and the solvent will affect PNA solubility. Crude PNAs tend to be less soluble than the purified product. Long PNAs are less soluble than shorter ones but the addition of one or more Lysine residues increases the solubility. PNAs have been used successfully at micromolar to millimolar concentrations. Some users have been surprised at the small amount of PNA required to react specifically.

12. Do you have suggestions for sequence design?

Yes. We have a three page sequence design guideline we would be happy to fax, mail or E-Mail to you. Basically, for the Duplex mode, PNA should be mixed purines and pyrmidines no longer than 18 bases (ideally alternating). For Triplex mode, the target DNA should be a homopurine sequence.

13. Is purification necessary for typical PNA applications?

Yes. For PNAs greater than a ten mer, you would probably want to purify the product or have us purify it for you. If you plan to work with a short PNA, and failure sequences will not interfere with your experiment, you can use the PNA unpurified. Purified PNAs tend to be more soluble than un purified PNAs.

14. What is the recommended purification method?

Standard peptide purification conditions are used to purify PNAs. A reverse phase C-18 column, fully end capped with a high carbon load (~10%), 150-300 Angstrom pore size, 5 micron particle size, works well. A shallow gradient of 0.5% to 1% per minute of water (A) and acetonitrile (B) each with a small amount of TFA (0.08% - .1%) as an organic modifier is commonly used.

15. Can I label PNA with radioactivity?

Yes, PNA can be labeled with radioactively by several different methods:

- For labeling with 32P, PNAs are made with the "Kemtide" peptide. This is then kinase labeled with gamma 32P ATP.
- For labeling with 125I, PNAs are made with a terminal Tyrosine which is then radioiodinated with 125I.
- Other approaches can be used by using PNAs with terminal Lysines (amino group) or Cysteines (thiol group) that can be labeled with radioactivity using standard methods.

16. Do any enzymes recognize PNA as a substrate?

No. None of the common proteases or nucleases have had any effect on PNA. Enzymes that require the sugar-phosphate backbone of DNA or RNA would not be expected to bind to PNA. No PNA enzymatic interactions have been found yet.

17. Can my in-house core facility make PNA for me?

Yes. We provide custom synthesis services, but we also sell Expedite PNA synthesis instruments and PNA synthesis reagents and for you to synthesize PNA in your lab. We are actively promoting the synthesis of PNAs by university core facilities.

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