

The Benefits of CleanAmp™ Technology for Less

CleanAmp™ Amidites allow you to manufacture CleanAmp™ Primers in-house using standard solid phase synthesis procedures. CleanAmp™ Amidites can be used on any DNA synthesizer. Make as much or as little material as you need, when you need it. Protocols for CleanAmp™ Primer synthesis can be found on pages 99-101. If followed precisely, it is very simple to prepare CleanAmp™ Primers.

Successful Syntheses with CleanAmp™ Amidites

Several properties of the CleanAmp™ Amidites and CleanAmp™ Primers are discussed below. Understanding these characteristics and carefully following the protocols provided will result in high quality CleanAmp™ Primers.

Thermolability of the Phosphorus Protecting Group

Devising a primer that would undergo Hot Start activation required the development of a phosphorus protecting group that would have the proper lability at 95°C. This had to be carefully balanced with the ability to prepare, deliver, store and handle the oligonucleotide. It is critical that the compound is handled appropriately and not subjected to elevated temperatures at any time.

Don't heat your CleanAmp™ Amidites or Primer above room temperature at any time during synthesis.
Don't use a centrifugal concentrator to dry your sample completely.

Base Lability of the Phosphorus Protecting Group

The CleanAmp™ Primer protecting group is a phosphotriester, like most other phosphorus protecting groups used for oligonucleotide synthesis. Phosphotriesters are labile to base, however the CleanAmp™ esters are much more stable than the β-cyanoethyl group commonly used. Therefore we readily identified conditions that removed the protecting groups from the rest of the oligonucleotide, while leaving the CleanAmp™ modification in place.

Do use fast deprotecting phosphoramidites.
Do use methanolic potassium carbonate at room temperature for deprotection.
Don't use standard phosphoramidites or standard deprotection schemes.

Use of DMSO as the Storage Solution

Although reasonable stability of CleanAmp™ Primers can be achieved in aqueous buffers, we were very fortunate to discover that DMSO is an excellent stabilizing solution, allowing us to store these modified primers at room temperature for extended times.

Do use pure DMSO.
Don't use the standard mix of ACN/aq. TEA (1:1) to elute your CleanAmp™ Primer from a reverse phase cartridge.

Assaying the Final Product

Once the CleanAmp™ Primer is eluted, an important part of the protocol is the HPLC assay of the product. For the best success in a PCR reaction, the amount of unprotected primer should be less than 1%.

Don't allow more than 1% unmodified material in the sample mixture, and in the case of Precision Primers, no more than 20% of the singly modified species.
Do assay all of your CleanAmp™ Primers by RP-HPLC to ensure that specifications are met.

For full details on successful CleanAmp™ Primer synthesis visit: www.trilinkbiotech.com/cleanamp.