

## Procedure for Synthesis of CleanAmp™ Primers

Reagents	Vendor
CleanAmp™ DNA phosphoramidites	TriLink BioTechnologies, Inc.
Fast deprotecting DNA phosphoramidites	Glen Research
Synthesis Column containing UnyLinker™	Glen Research
Acetonitrile, dried to ≤ 20 ppm water (ACN)	Fisher Scientific
Dichloromethane	Fisher Scientific
Activated Molecular Sieves	TriLink BioTechnologies, Inc.
Cap A reagent with Phenoxyacetic anhydride	Glen Research
Cap B reagent with N-Methyl-Imidazole	Glen Research
HPLC Grade Water	Fisher Scientific
50 mM Potassium carbonate in Methanol	Glen Research
1M TEAA, pH 7.2 (Triethylammonium Acetate)	TriLink BioTechnologies, Inc.
2% TFA in water (Trifluoroacetic Acid)	TriLink BioTechnologies, Inc.
DMSO anhydrous	Fisher Scientific

### Procedure:

#### A. Synthesis of CleanAmp™ primers on solid support at 1 μmole scale :

1. Prepare solutions of each fast deprotecting phosphoramidite monomer in anhydrous acetonitrile (ACN) using manufacturer's instructions. We recommend using a concentration of 0.067 M. **Higher concentrations may lead to precipitation of the amidite.**
2. Prepare 0.067 M solutions of each CleanAmp™ Amidite (except for CleanAmp™ -dG) in dry acetonitrile in a standard DNA synthesizer bottle. Prepare 0.067 M solution of CleanAmp™-dG phosphoramidite monomer in mixture of dry dichloromethane (DCM):ACN (1:1) in a standard DNA synthesizer bottle.

To properly dissolve a 100 μmole bottle of a CleanAmp™ Amidite to obtain a 0.067 M solution, use 1.50 mL of the appropriate solvent.

3. Add activated molecular sieves (20 beads/mL) to each amidite bottle, flush with dry argon gas, recap the bottle, seal using parafilm and keep it overnight at room temperature before use.
4. Purchase, or manually fill, a 1 μmole synthesis column with UnyLinker™ support.
5. Load the DNA synthesizer with the reagents listed in the Reagents table in the appropriate well according to manufacturer's instructions.
  - a. Load the amidites. Use a spare port for the CleanAmp™ Amidite(s).
  - b. Load the ancillary reagents. It is critical that the appropriate capping reagents are used for fast deprotecting monomers. "Cap A" must be the phenoxyacetyl anhydride version of the reagent. **DO NOT USE ACETIC ANHYDRIDE.**
6. Follow an automated synthesis protocol for fast deprotecting phosphoramidite monomers as recommended by the instrument manufacturer with the following exception: increase the coupling time for CleanAmp™ Amidites to ten minutes.
7. At the last coupling cycle, leave the DMT group "On".
8. When synthesis is completed, dry the column using argon flow.
9. It is recommended to proceed with the cleavage/deprotection of CleanAmp™ Primer immediately after completion of the synthesis, otherwise keep the dry column at -20°C.

#### B. Cleavage/Deprotection of CleanAmp™ Primers:

1. Transfer support (with oligonucleotide) from the column to a screw cap 8 mL glass vial.
2. Add 6 mL of freshly prepared 50 mM potassium carbonate solution in MeOH.
3. Place the vial on a rotary mixer for 20 hrs at room temperature.
4. Let support settle and transfer supernatant solution into a 50 mL conical tube.
5. Wash support with 2 mL of 1 M Triethylammonium Acetate (TEAA) and add to the deprotection solution.
6. Measure the total crude yield using UV spectrophotometer.
7. Process the solution immediately using SepPak isolation/purification, otherwise keep the solution at -80°C to -20°C.

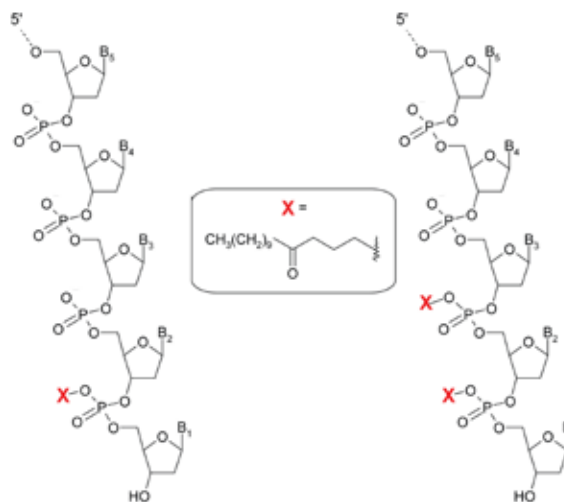
#### C. SepPak isolation and purification procedure:

1. Fit a 60 mL syringe to SepPak cartridge; part # 20515.
2. Equilibrate cartridge with 10 mL of acetonitrile, followed by 10 mL of 50% acetonitrile in 100 mM TEAA and finally by 15 mL of 25 mM TEAA.

3. Prepare sample for loading:
  - a. Add 2 mL of 1 M TEAA to the sample and pipette the solution into 5 mL syringe fitted with a luer lock and a 0.44 micron filter disc. Filter the solution into a 50 mL conical tube.
  - b. Rinse the deprotection vessel with another 2 mL of water, passing that through the filter and into the tube with the sample.
  - c. Dilute the solution to 50 mL with 1 M TEAA.
4. Load the solution to cartridge with a flow rate of 1-2 mL/min and pour into the 60 mL loading syringe on cartridge. Collect and read the absorbance of the flow-through to ensure the primer is bound to the cartridge.
5. Rinse the cartridge with 20 mL of water.
6. Pass 10 mL of 2% TFA through the cartridge over 3 minutes. Observe appearance of orange colored band.
7. Immediately rinse SepPak with 20 mL HPLC water.
8. Pass 10 mL of 1 M TEAA through the cartridge over 5 minutes to neutralize acid.
9. Wash SepPak with a purification buffer:
  - a. For CleanAmp™ Turbo Primers, apply 15 mL of 15% ACN, 100 mM TEAA to cartridge. Collect rinse.
  - b. For CleanAmp™ Precision Primers, apply 15 mL of 25% ACN, 100 mM TEAA to cartridge. Collect rinse.
10. Rinse cartridge with 20 mL of water. Collect rinse.
11. Elute samples using DMSO.
  - a. Attach a 3mL slip tip syringe to the cartridge.
  - b. Add 200 µL DMSO to syringe and elute into a microtube, labeled fraction 1.
  - c. Remove syringe from cartridge, pull out plunger, reattach the syringe to the cartridge, and push air into same graduated tube to completely remove all the DMSO.
  - d. Add 200 µL DMSO to syringe and elute into a fresh microtube (fraction 2) using plunger. Add another 200 µL to DMSO to syringe and elute into the same graduated tube for a total of 400 µL DMSO in fraction 2.
  - e. Add 200 µL DMSO to syringe and elute into a fresh microtube (fraction 3).
12. Cap, vortex and spin down fractions. Read fraction 2 only. If yield is less than 10 OD<sub>260</sub> units, read the absorbance of fraction 3. Add just enough of the material in fraction 3 to the material in fraction 2 to achieve 10 OD<sub>260</sub> units.
13. Determine molar concentration of purified primer in DMSO solution using the absorbance reading and the calculated extinction coefficient. If necessary, dilute with DMSO to obtain a 0.200 mM solution.
14. Analyze purified CleanAmp™ Primers by RP-HPLC to ensure that no more than 1-2% of unmodified primer is in the purified sample, and no more than 20% of the singly modified species if a Precision Primer was prepared.
15. Store the primer at 4°C or less. Although it is very stable at room temperature in the DMSO solution, long term storage is improved at lower temperatures.

## HPLC Analysis of CleanAmp™

1. Analyze a 0.2 OD<sub>260</sub> unit sample of the isolated material by reverse phase HPLC to determine the level of contamination by primers not protected by the CleanAmp™ moiety.
2. Method:
  - a. Column: Waters µBondapak 8 mm RP cartridge (WAT 027324) or comparable.
  - b. Buffer A: 100 mM TEAA; Buffer B: ACN
  - c. Gradient: 0-100% B over 40 minutes
  - d. Flow Rate: 1 mL/minute
  - e. Observe at 260 nm.
3. Determine the mobility of the unprotected primer by heating another 0.2 OD<sub>260</sub> unit sample for 40 minutes at 95°C, which will completely remove the CleanAmp™ modification from the primer.
4. Turbo should have less than 1% of the fully deprotected primer while Precision should contain less than 2% of that contaminant. Precision should have less than 20% of the singly modified material, which elutes between the unmodified product and the doubly modified Precision Primer.
5. If specifications are not met, the benefits of the CleanAmp™ technology may still be experienced. The system is very robust and some PCR applications are less demanding than others.



CleanAmp™ Turbo Primers  
Single 4-oxo-tetradecyl modification

CleanAmp™ Precision Primers  
Double 4-oxo-tetradecyl modification

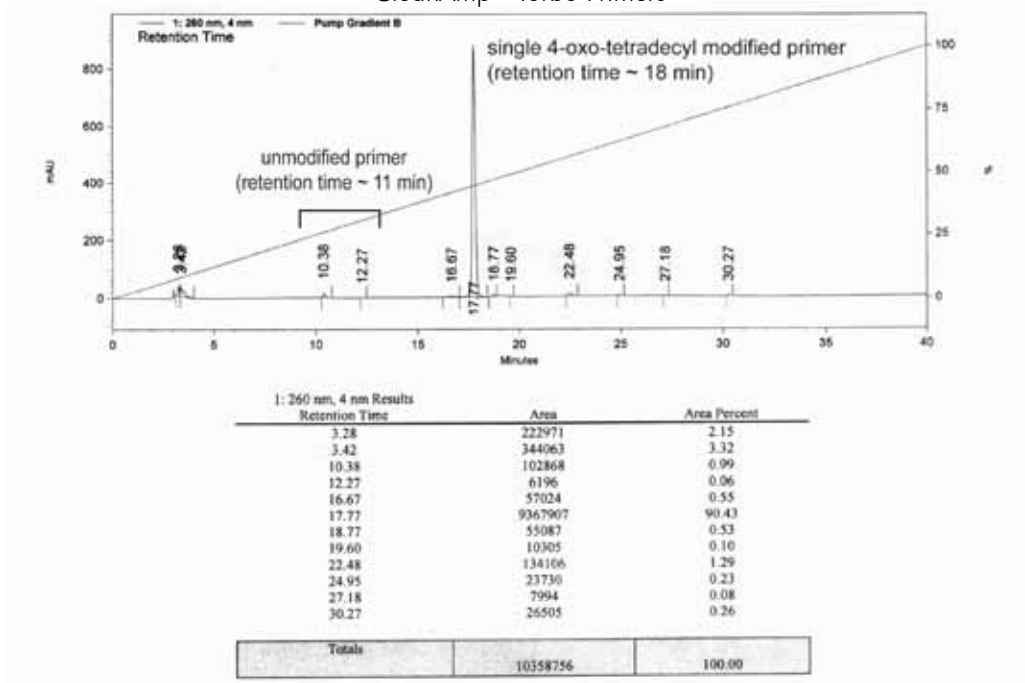
For the most current CleanAmp™ Primer protocol visit:  
[www.trilinkbiotech.com/cleanamp](http://www.trilinkbiotech.com/cleanamp).

\*See next page for representative RP-HPLC analysis of CleanAmp™ Turbo and Precision Primers.

Reverse-phase HPLC analysis:  
CleanAmp™ Primers

RP3-HPLC : Waters uBondapak C18, 10 μm, 125 A, 300x3.9 mm  
 Buffer A : 100 mM TEAA pH = 7-7.5      Buffer B : Acetonitrile  
 Gradient : 0-100% B over 40 min      Flow Rate : 1 mL/min      Temperature : RT  
 Injection : 30 μL

CleanAmp™ Turbo Primers



CleanAmp™ Precision Primers

