



# CleanAmp™ Primers

The Next Generation in Hot Start PCR

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## Product Manual

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# Contents

Storage and Handling	2
Recommended Solution Storage Temperatures	2
Product Introduction	2
Selection of CleanAmp™ Primer Type and Purity	3
Important Reaction Parameters	4
Choice of DNA Polymerase	5
Use with Commercially Available Master Mixes	5
Protocols for Taq DNA Polymerase	6
Standard Thermal Cycling	6
Fast Thermal Cycling	6
Multiplexed	6
One-Step Reverse Transcriptase PCR	8
Real-Time	11
Troubleshooting	12
References	13
Contact Information	14

## Storage and Handling

CleanAmp™ Primers should be stored at –20°C to avoid loss of Hot Start activity. CleanAmp™ Turbo and CleanAmp™ Precision Primers are supplied in a DMSO stock solution. Each oligo is guaranteed to be 10-15 OD ( $A_{260}$ ) at a concentration of at least 200  $\mu\text{M}$ <sup>1</sup>. It is recommended that stock solutions be divided into several tubes upon receipt to prevent extended room temperature exposure over many uses.

Dilutions of the stock solution should be made as needed, using water or aqueous buffer (pH 7-9) as the diluent and stored on ice. For maximal performance the working solution should be used immediately. Any remainder should be discarded.

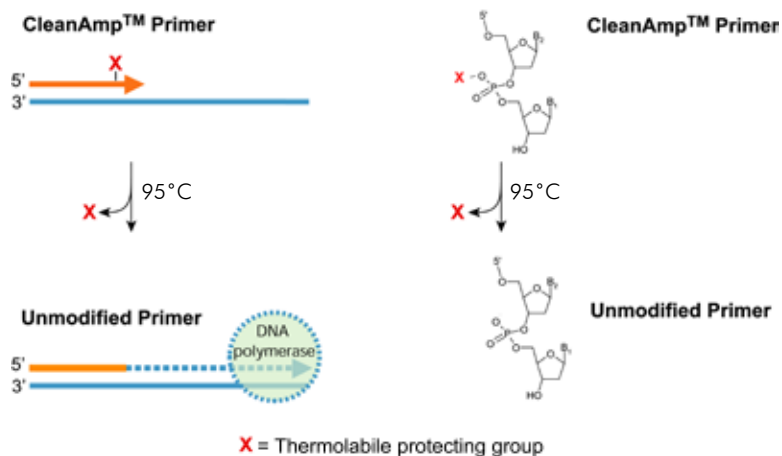
### Recommended Solution Storage Temperatures

	Room temp.	4°C	-20°C
Stock solution	48 hrs	60 days	1 year
Working solution	3 hrs	3 days	20 days

## Product Introduction

Our CleanAmp™ Products offer a complete chemical solution to Hot Start activation through two widely ignored components of the PCR reagent mix: the dNTPs and the Primers. CleanAmp™ Primer modifications can be used with any PCR polymerase, introduced into any sequence and are effective in a variety of PCR-based applications. CleanAmp™ Primers contain chemical modifications that allow for primer-based Hot Start activation in PCR (1). This modification prevents primer extension at the lower temperatures of PCR set-up and manipulation. A Hot Start thermal activation step removes the modification and generates the corresponding unmodified primer, which supports amplification of the desired target. CleanAmp™ Primers specifically amplify your target by

eliminating extension off of primer dimer and mis-priming events. Furthermore, CleanAmp™ Primers are compatible with a number of standard DNA polymerases, such as Taq DNA polymerase, eliminating the need for Hot Start DNA polymerases.



### Selection of CleanAmp™ Primer Type and Purity

Two types of CleanAmp™ Primers are available. CleanAmp™ Turbo and Precision Primers differ in the rate of thermal activation. CleanAmp™ Turbo Primers activate more quickly than CleanAmp™ Precision Primers. The differential rate of activation of the two varieties is beneficial for many applications and PCR needs.

CleanAmp™ Turbo Primers	CleanAmp™ Precision Primers
Fast cycling (2 step protocol)	Standard cycling (3 step protocol)
Multiplexed PCR	One-step reverse-transcription PCR (RT-PCR)
Improved amplicon yield	Improved specificity of amplification and limit of detection
Reduced mis-priming/primer dimer formation	Greatest reduction in mis-priming/primer dimer formation

CleanAmp™ Primers are commonly offered as cartridge-purified. The cartridge purification method enriches for the CleanAmp™ modification, with some enrichment of full-length sequences. Cartridge-purified material offers the benefits of a Hot Start activation approach, without the extra cost of HPLC purification. All CleanAmp™ Primers undergo quality control analysis by PAGE, MS and RP-HPLC to ensure the highest quality.

CleanAmp™ Primers can also be purchased as HPLC-purified upon request. HPLC-purified material is best suited for high fidelity applications, where PCR performance is of the utmost importance. We recommend doing all of your development work with cartridge-purified primers, then transitioning to HPLC-purified primers once all reaction parameters have been identified.

### Important Reaction Parameters

**Initial Denaturation Time:** 2-10 minutes at 95°C is recommended for standard thermal cycling protocols. If using a chemically modified DNA polymerase, such as AmpliTaq Gold™ DNA polymerase with CleanAmp™ Primers, an initial denaturation of 10-20 minutes may be required. For the modified conditions of fast cycling, shorter denaturation times at 94°C can be employed (see PCR Protocols).

**Primer Concentration:** CleanAmp™ Primers can be employed over a wider range of primer concentration than unmodified primers, while still providing reduction in off-target amplification products. We recommend performing a titration of your primers upon receipt to determine the optimal concentration for increased target amplicon formation with reduction of non-specific products. In general, primer/template systems that are prone to mis-priming require a much lower primer concentration than systems prone to primer dimer formation.

**Use of DMSO:** DMSO is a commonly used additive in PCR that has been used to improve the efficiency of PCR by disrupting base pairing in GC rich sequences. It has been shown that the addition of DMSO improves PCR yield and specificity (2, 3, 4). Typically, between 0-10%

DMSO can be employed in PCR, however in some instances, DMSO may cause PCR inhibition. Be sure that the percent DMSO that will be introduced into your reactions by the CleanAmp™ Primers will not be inhibitory to your PCR. At high percentages, DMSO has been found to reduce the performance of CleanAmp™ Primers by slowing down the rate of release of the protecting group. When using CleanAmp™ Primers, we recommend the addition of DMSO as a reaction supplement only if absolutely necessary and to a maximum of 2% (vol/vol).

### Choice of DNA Polymerase

Taq DNA polymerases validated for use with CleanAmp™ Primers:

DNA Polymerase Name	Vendor
Taq DNA polymerase (native and recombinant)	Invitrogen
Taq DNA polymerase (recombinant)	New England Biolabs
EconoTaq® DNA polymerase	Lucigen

DNA polymerases validated for use with CleanAmp™ Primers. PCR performances are comparable to reactions containing Taq.

DNA Polymerase Name	Vendor
Pfu DNA polymerase (exo + and exo -)	Stratagene
DyNAzyme™ DNA polymerase	Finnzymes
Tth DNA polymerase	USB
Tfi DNA polymerase	Invitrogen
Deep Vent <sub>r</sub> ™ DNA polymerase	New England Biolabs

### Use with Commercially Available Master Mixes

It is not recommended that CleanAmp™ Primers be employed with commercially available PCR master mixes. The presence of additives in these mixes has been found to slow down the rate of release of the CleanAmp™ modification group. In many cases this results in PCR inhibition. Please refer to the PCR Protocols section for recommended usage of CleanAmp™ Primers.

## Protocols for Taq DNA Polymerase

### Standard Thermal Cycling

### Fast Thermal Cycling

### Multiplexed

### Components

- Taq DNA Polymerase (5 U/μL) (Invitrogen)
- 10X PCR buffer (10 mM Tris-HCl, 50 mM KCl, pH 8.3 at 25°C; Invitrogen)
- 50 mM Magnesium Chloride (Invitrogen)
- 10 mM dNTPs- deoxynucleotide triphosphates (New England Biolabs)
- CleanAmp™ Primers
- Sterile de-ionized water

### Protocol

The following protocol is for the set up of a 50 μL reaction. The reaction volumes can be scaled accordingly for your desired final volume.

1. For all components except CleanAmp™ Primers and DNA polymerase, thaw the reaction components, vortex to mix, centrifuge briefly and store on ice.
2. For the CleanAmp™ Primers:
  - a. Thaw the DMSO stock solution.
  - b. After thawing, vortex and pulse centrifuge two to three times to thoroughly mix.
  - c. Remove an aliquot of the DMSO stock solution for addition into the master mix. If needed, dilute CleanAmp™ Primers with water or buffer (pH 7-9) to the desired working concentration. For maximal performance, dilutions should be used immediately and any remainder discarded.
3. We recommend making a master mix containing all components except for the DNA template sample. Add each component as shown in the following table (multiply amounts by the number of reactions needed) into a microcentrifuge tube on ice.
4. Mix gently by pipetting up and down. Pulse spin if needed.

Component	Final Concentration (in a 50 $\mu$ L reaction)	Volume for 1 reaction	Volume for 10 reactions
Sterile de-ionized water	-	Up to 45 $\mu$ L	Up to 450 $\mu$ L
10X PCR buffer	1X	5 $\mu$ L	50 $\mu$ L
<b>50 mM Magnesium Chloride</b>			
Standard Thermal Cycling	2.5 mM	2.5 $\mu$ L	25 $\mu$ L
Fast Thermal Cycling	5.0 mM	5.0 $\mu$ L	50 $\mu$ L
Multiplexed	5.0 mM	5.0 $\mu$ L	50 $\mu$ L
<b>dNTP mixture (10 mM of each)</b>			
Standard Thermal Cycling	0.2 mM	1 $\mu$ L	10 $\mu$ L
Fast Thermal Cycling	0.8 mM	4 $\mu$ L	40 $\mu$ L
Multiplexed	0.4 mM	2 $\mu$ L	20 $\mu$ L
<b>CleanAmp™ Forward/Reverse Primer<sup>2</sup></b>			
Standard Thermal Cycling	0.1-0.5 $\mu$ M each	Variable	Variable
Fast Thermal Cycling	0.5-2.0 $\mu$ M each	Variable	Variable
Multiplexed	0.2-1.0 $\mu$ M each	Variable	Variable
<b>DNA polymerase (variable units/<math>\mu</math>L)</b>			
Standard Thermal Cycling	0.025 units/ $\mu$ L	Variable	Variable
Fast Thermal Cycling	0.1-0.2 units/ $\mu$ L	Variable	Variable
Multiplexed	0.05-0.1 units/ $\mu$ L	Variable	Variable
<b>Total Volume (<math>\mu</math>L)</b>	-	<b>45 <math>\mu</math>L</b>	<b>450 <math>\mu</math>L</b>

- Aliquot 45  $\mu$ L of master mix into each thin-walled PCR tube.
- Add 5  $\mu$ L of template DNA into each reaction for a final reaction volume of 50  $\mu$ L.
- Pulse spin PCR tubes to remove bubbles and collect reaction solution at the bottom of the tube.

- Place the tubes into a thermal cycler with a heated lid and perform the appropriate cycling conditions.

For standard and multiplexed thermal cycling conditions:

95°C for 2-10 min  
 [95°C for 30 sec; 48-60°C<sup>3</sup> for 30 sec; 72°C for 0.5-2 min]  
 25-50 cycles  
 72°C for 10 min

For fast thermal cycling conditions:

[95°C for 10 sec; 62-72°C<sup>4</sup> for 30 sec] 40-50 cycles

- Analyze the reaction components by agarose gel electrophoresis.

### Use with different DNA polymerases

For reactions containing *Pfu* DNA polymerase (exo + and exo - ), DyNAzyme™ DNA polymerase, *Tth* DNA polymerase, *Tfi* DNA polymerase or Deep Vent<sub>R</sub>™ DNA polymerase, each polymerase should be used with the corresponding buffers provided by the manufacturer. The reaction conditions are identical to those for *Taq* DNA polymerase, with the exception of Deep Vent<sub>R</sub>™ DNA polymerase, which requires 2.5 units of DNA polymerase, rather than 1.25 units.

### One-Step Reverse Transcriptase PCR

#### Components

- *Taq* DNA Polymerase (5 U/ $\mu$ L) (Invitrogen)
- M-MLV Reverse Transcriptase (200 U/ $\mu$ L) (Invitrogen)
- RNase Inhibitor (40 U/ $\mu$ L) (Ambion)
- 10X PCR buffer (Invitrogen)
- 50 mM Magnesium Chloride (Invitrogen)
- 10 mM dNTP mixture - deoxynucleotide triphosphates (New England Biolabs)
- Sterile de-ionized water
- PolydT primer or random primer
- CleanAmp™ Precision Primers

## Protocol

1. For all components except CleanAmp™ Precision Primers and enzymes (i.e. RT enzyme, DNA polymerase, RNase Inhibitor), thaw, vortex to mix, centrifuge briefly and store on ice.
2. For the CleanAmp™ Precision Primers:
  - a. Thaw the DMSO stock solution.
  - b. Vortex and pulse centrifuge two to three times to thoroughly mix the stock solution then place on ice.
  - c. Remove an aliquot of the DMSO stock solution for addition into the master mix. If needed, dilute CleanAmp™ Primers with water or buffer (pH 7-9) to the desired working concentration. For maximal performance, dilutions should be used immediately and any remainder discarded.
3. It is recommended to make a master mix containing all components except for the RNA template sample. Add each component as shown in the following table (multiply amounts by the number of reactions needed) into a microcentrifuge tube on ice.
4. Mix the master mix gently by pipetting up and down. Pulse spin if needed.
5. Aliquot 24  $\mu\text{L}$  of master mix into each thin-walled PCR tube.
6. Add 1  $\mu\text{L}$  of Total RNA or Poly(A)+ RNA sample (0.1-1  $\mu\text{g}$ ) into each reaction for a final reaction volume of 25  $\mu\text{L}$ . Note: should the volume of RNA template vary from 1  $\mu\text{L}$ , adjust the final volume of master mix accordingly to achieve a final reaction volume of 25  $\mu\text{L}$ .
7. Pulse spin PCR tubes to remove bubbles and collect reaction solution at bottom of tube.
8. Place the tubes into a thermal cycler with a heated lid.
9. Cycle using standard RT-PCR thermal cycling conditions:
  - 42°C for 30 min, 95°C for 10 min
  - [95°C for 30 sec; 48-60°C<sup>3</sup> for 30 sec; 72°C for 30 sec]
  - 30-40 cycles
  - 72°C for 5 min (Can omit if performing real-time experiment)
10. Analyze by agarose gel electrophoresis.

Component	Final Concentration (in a 25 $\mu\text{L}$ reaction)	Volume for 1 reaction	Volume for 10 reactions
Sterile de-ionized water	-	Up to 24 $\mu\text{L}$	Up to 240 $\mu\text{L}$
10X PCR buffer	1X	2.5 $\mu\text{L}$	25 $\mu\text{L}$
50 mM Magnesium Chloride	1.5 mM	0.75 $\mu\text{L}$	7.5 $\mu\text{L}$
dNTP mixture (10 mM of each)	0.16 mM	0.4 $\mu\text{L}$	4 $\mu\text{L}$
PolydT or random primer (50 $\mu\text{M}$ )	1 $\mu\text{M}$	0.5 $\mu\text{L}$	5 $\mu\text{L}$
RNase Inhibitor (variable units/ $\mu\text{L}$ )	2.5 units	Variable	Variable
CleanAmp™ Precision Forward Primer	0.1-0.5 $\mu\text{M}$	Variable	Variable
CleanAmp™ Precision Reverse Primer	0.1-0.5 $\mu\text{M}$	Variable	Variable
Reverse Transcriptase (Variable units/ $\mu\text{L}$ )	1 unit/ $\mu\text{L}$	Variable	Variable
DNA polymerase <sup>5</sup> (variable units/ $\mu\text{L}$ )	0.0125 units/ $\mu\text{L}$	Variable	Variable
<b>Total Volume (<math>\mu\text{L}</math>)</b>	-	<b>24 <math>\mu\text{L}</math></b>	<b>240 <math>\mu\text{L}</math></b>

## Notes

- <sup>1</sup> See Certificate of Analysis for exact yield and concentration.
- <sup>2</sup> In your initial experiments, we recommend performing a titration of primer concentration for optimal performance. Please note: the optimal concentration for CleanAmp™ Primers is not necessarily the same as for unmodified primers.
- <sup>3</sup> The annealing temperature should be chosen for optimal PCR performance. Most primer design software provides users with a recommended annealing temperature. Otherwise, the annealing temperature can also be optimized experimentally either by using a thermal cycler with gradient functionality or by performing several sequential experiments in which the annealing temperature is varied.
- <sup>4</sup> Use the following calculation for the annealing/extension temperature: Annealing/extension temperature (°C) = (72°C + average T<sub>m</sub> of primers)/2
- <sup>5</sup> The amount of DNA polymerase can be increased by as much as four-fold over the amount described in the protocol to achieve more robust amplicon formation.

## Real-Time

The standard, multiplexed and fast cycling protocols on the previous pages can be adapted for real-time experiments with the following alterations to the protocol:

### Additional real-time components:

#### SYBR Green® detection:

- o SYBR Green® I nucleic acid stain (10,000X) (Invitrogen)
- o Passive reference ROX dye (1 mM) (Agilent)

#### TaqMan® Probe detection

- o TaqMan® Probe
- o Passive reference ROX dye (1 mM) (Agilent)

**Adaptation to real-time PCR:** The reaction described on the previous page should be identical, except for the following additions for SYBR Green®- or TaqMan® Probe-based detection. Reactions should be incubated in a thermal cycler capable of real-time detection, where fluorescence data can be collected at the completion of the annealing step of each cycle.

- o For SYBR Green®-based detection, 30 or 300 nM passive ROX reference dye (dependent on real-time instrument manufacturer, please reference Agilent’s manual for details) and 0.15X SYBR Green® I nucleic acid stain should be included in the reaction.
- o For TaqMan® Probe -based detection, 30 or 300 nM passive ROX reference dye (dependent on real-time instrument manufacturer, please reference Agilent’s manual for details) and 50-20 nM TaqMan® Probe should be included. The optimal TaqMan® Probe concentration should be determined by performing serial dilutions and identifying the concentration that provides the earliest C<sub>q</sub> and maximal fluorescence intensity.

**Note:** The dilution of passive ROX reference dye can be kept at 4°C for approximately one month. For both the passive ROX reference

dye and TaqMan® Probe, keep tubes protected from light at all times (i.e. wrap tubes with aluminum foil).

**Note:** Reactions should be set up in the appropriate optical tubes recommended by the instrument manufacturer. Wear powder-free gloves when performing all real-time set-ups. Optical tubes and caps should be wiped with a Kimwipe® to remove any residue that may interfere with optical reading.

## Troubleshooting

Observation	Probable Cause	Suggestion(s)
No amplification product or low amplicon yield	Insufficient activation of CleanAmp™ Primers during thermal cycling	Increase the concentration of CleanAmp™ Primers to up to 2μM
		Optimize the duration of the initial denaturation time to up to 20 minutes when used in conjunction with chemically-modified DNA polymerases or PCR master mixes.
		Verify that the level of DMSO that was introduced into the reaction by CleanAmp™ Primers is not inhibitory to the reaction.
	Thermal cycling protocol is not optimized	If a commercially available PCR master mix is being employed, attempt experiments using the appropriate set-up from the PCR Protocols section.
		Increase extension time. Generally extension times should be 1-2 minutes per kb of target.
		Increase the number of thermal cycles in 5 cycle increments.
Problem with reagents or reaction conditions	Optimize annealing temperature.	
	Prepare fresh reagents, including reaction buffer and dNTPs.	
	Verify that template is good in quality and of sufficient quantity.	
Non-Specific product formation	Excessive off-target primer extension	Verify primer design to ensure adequate complementarity to the DNA target.
		Optimize the MgCl <sub>2</sub> concentration.
		Titrate the concentration of the CleanAmp™ Primers to as low as 50 nM.
		Reduce the extension time to avoid spurious amplification products.
		Reduce the amount of DNA polymerase.
		Titrate the amount of template DNA.

## References

1. Lebedev, A.V, Paul, N., et al. (2008) Nucleic Acids Res. 36, e131.
2. Frackman, S., Kobs, G. (1988) Promega Notes Number 65, 27.
3. Winship, P.R. (1989) Nucleic Acids Res. T17, 1266.
4. Chakrabarti, R. & Schutt, C.E. Nucleic Acids Res. 29, 2377-2381.

## Contact Information

For further information on CleanAmp™ Primers, please visit our website or contact



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Version I, June 2009

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