Ampliqon

UniPOL

For Long Range PCR

With 10X UniPOL Buffer A ($MgCl_2$ 15mM)
With 10X UniPOL Buffer B ($MgCl_2$ 15mM)

Cat. No.: 270701 (100 Reactions)

Cat. No.	Reactions	10X UniPOL Buffer A ($MgCl_2$ 15mM)	10X UniPOL Buffer B ($MgCl_2$ 15mM)	DMSO
270701	100	1.5 mL	1.5 mL	0.5 mL
270702	200	1.5 mL	1.5 mL	0.5 mL
270703	500	1.5 mL	1.5 mL	1.5 mL
270704	1000	2x 1.5 mL	2x 1.5 mL	1.5 mL
270706	2.500	4x 1.5 mL	4x 1.5 mL	1.5 mL

Store at -20°C. *Reagent for in-vitro laboratory use only*

General Description

Ampliqon UniPOL-Long Range PCR Enzyme mix, combines Ampliqon's high quality *Taq* DNA Polymerase with a small amount of AccuPOL, which exhibit 3'→5' proofreading exonuclease activity. The UniPOL Enzyme mix is optimised for amplifications of 5-20 kb with a high yield.

Amplification using *Taq* DNA Polymerase is generally limited to amplifying up to 4-6 kb, depending on template. This is partly due to the lack of 3'→5' proofreading exonuclease activity of the *Taq* DNA Polymerase. Mis-incorporation of nucleotides often leads to processive mistakes and consequently a terminal event will occur and the elongation will arrest. A mix of the processive *Taq* DNA Polymerase and the AccuPOL proofreading enzyme, which corrects mis-incorporated nucleotides, increases the length of the amplification product.

Due to differences in complexity of template, Ampliqon has optimized two different Buffers- UniPOL Buffer A and UniPOL Buffer B.
Some difficult templates require additives, such as DMSO, which has been reported to give DNA thermal stability against depurination and to be useful for G-C rich templates.

10X UniPOL Buffer A

Tris-HCl pH 8.9, KOAC, 15 mM $MgCl_2$,

10X UniPOL Buffer B

Tris-HCl pH 8.9, KOAc, 15mM $MgCl_2$, and Stabilizer

Unit Definition

One unit is defined as the amount that incorporates 10 nmoles of dNTPs into acid-precipitable form in 30 minutes at 72°C under standard assay conditions.

Storage and Dilution Buffer

Enzyme is supplied in 20 mM Tris-HCl pH 8.3, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5% Tween 20®, 0.5% NP40, 50% glycerol.

Quality Control

Each lot of UniPOL is tested for contaminating activities, with no trace of endonuclease activity, nicking activity, exonuclease activity or priming activity.

Suggested Protocol using UniPOL-Long Range PCR

This protocol serves as a guideline for primer extensions. Optimal reaction conditions such as incubation times, temperatures, and amount of template DNA may vary and must be individually determined.

Notes:

- Set up reaction mixtures in an area separate from that used for DNA preparation or product analysis.
- 15mM $MgCl_2$ is present in the 10X buffers. The 1X concentration is 1.5mM $MgCl_2$.
- Addition of DMSO to a final concentration of 1-5% may increase yield and improve reliability of the system for some complex PCR targets.
- Effective denaturation can be accomplished by the use of higher temperature for shorter periods of time or by adding DMSO.
- Reliable amplification of long DNA sequences requires:
  1) Effective denaturation of DNA template
  2) Protection from depurination.
  3) Extension time long enough to produce large products.

  (1) and 2) can be achieved with addition of DMSO, if necessary).

1. Thaw 10x UniPOL Buffer A and/or 10x UniPOL Buffer B, dNTP mix, primer solutions. **It is important to mix the solutions completely before use to avoid localized concentrations of salts**.
2. Prepare a master mix according to Table 1. The master mix typically contains all the components needed for extension except the template DNA.

Table 1. ***Reaction components (master mix and template DNA)***

Component	Vol./reaction	Final Conc.
10X UniPOL Buffer A or 10x UniPOL Buffer B	5 µL	1X
dNTP mix (12.5 mM of each)	0.8 µL	0.2 mM of each dNTP
Primer A	Variable	0.1–1.0 µM
Primer B	Variable	0.1–1.0 µM
UniPOL Long Range PCR	0.5µL	
Distilled Water	Variable	- - - -
Template DNA	Variable	Variable
Optional: DMSO (100%)	0.5µL – 2.5 µL	1 – 5%
TOTAL volume	50 µL	- - - -

Table 2. *$MgCl_2$ concentration in a 50 µL reaction*

Final $MgCl_2$ conc. in reaction (mM)	1.5	2.0	2.5	3.0	3.5	4.0	4.5
Additional volume of 25 mM $MgCl_2$ per reaction (µL):	0	1	2	3	4	5	6

3. Mix the master mix thoroughly and dispense appropriate volumes into reaction tubes. Mix gently, *e.g.*, by pipetting the master mix up and down a few times.
4. Add template DNA to the individual tubes containing the master mix.
5. Program the thermal cycler according to the manufacturer's instructions.

   For maximum yield and specificity, temperatures and cycling times should be optimized for each new template target or primer pair.

Both two and three step PCR can be used;

Ex. Three step PCR

25-40 cycles:

95°C	10-30 sec.	*Denature template*
55-68°C	10-30 sec.	*Anneal primer*
72°C	1-20 min.	*Elongation****

72°C for 10 min. *Final Elongation*

***Recommended time is approx. 45 sec. per kb of target*

Ex. Two step PCR

25-40 cycles:

95°C	10-30 sec.	*Denature template*
68°C	10-30 sec.	*Anneal primer & Elongation ****

72°C for 10 min. *Final Elongation*

***Recommended time is approx. 45 sec. per kb of target*

6. Place the tubes in the thermal cycler and start the reaction.

Related Products

Description	Cat. No.
Taq DNA Polymerase (500 Units) *with 10X Ammonium Reaction Buffer with 10X Standard Reaction Buffer*	**110303**
Taq DNA Polymerase (500 Units) *with 10X Combination Buffer*	**110403**
Taq DNA Polymerase (500 Units) *with 10X Mg^{++} Free Ammonium Buffer*	**110503**
Taq DNA Polymerase 2.0X Master Mix (100 Reac) *with 2.0 mM MgCl2*	**150301**
Taq DNA Polymerase 2,0X MaMi RED (100 Reac) *with 1.5 mM MgCl2,*	**180301**
Taq DNA Polymerase 2.0X MaMi RED (100 Reac) *with 2.0 mM MgCl2*	**190301**
AccuPOL DNA Polymerase (500 Units)	**210303**
TEMPase Hot Start DNA Polymerase (500Units) *with 10X TEMPase Buffer I with 10X TEMPase Buffer II*	**220303**
UniPOL –Long Range PCR (100 Reac)	**270701**
Rapid Ligation Kit (50 React)	**750300**
RT-PCR One Tube (100 Reac)	**740301**
TEMPase Hot Start 2X Master Mix *with TEMPase Buffer I* (100 Reac)	**230301**
TEMPase Hot Start 2X Master Mix *with TEMPase Buffer II* (100 Reac)	**230701**
dNTP Mix (2 x 500µl) (12.5 mM of each dA, dC, dG and dT)	**501004**
dNTP Mix, (2 x 500 µl) (10 mM of each dA, dC, dG and dT),	**502004**
GC5 Value Efficiency, 10^8 Cfu/µg pUC19 Chemically Competent Cells, (10x 200µl)	**812010**
GC5 High Efficiency, 10^9 Cfu/µg pUC19 Chemically Competent Cells, (10x 50µl)	**805010**
GC5 High Efficiency, 10^9 Cfu/µg pUC19 Chemically Competent Cells, (5x 200µl)	**802005**
SuperPath GC10, 10^{10} Cfu/µg pUC19 ElectroCompetent Cells, (5x 80µl)	**830805**
SOC Medium, 10x 10mL	**800000**

NOTICE
In certain countries, patents cover the PCR process. This product is intended for researchers having a license to perform PCR or those not required to obtain a license.