Ampliqon
Bioreagents & PCR Enzymes

Taq RedPOL DNA Polymerase

Concentration: 5 units/µL

Catalogue no.: 200309 (20000 units)

Cat. No.	Size Units	10X Ammonium Buffer ($MgCl_2$ 15mM)	10X Standard Buffer ($MgCl_2$)	$MgCl_2$ 25 mM
200303	500	1.5 mL	1.5 mL	1.5 mL
200304	1000	2x 1.5 mL	2x 1.5 mL	2x 1.5 mL
200306	2500	4x 1.5 mL	4x 1.5 mL	4x 1.5 mL

(other quantities available)

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

General Description

Ampliqon Taq RedPOL DNA Polymerase is a thermostable recombinant DNA polymerase, which exhibits very high activity in primer extension and other molecular biology applications. Taq RedPOL contains a red dye which provides easy and quick identification of reactions to which enzyme was added and allows confirmation of complete mixing. The inert dye has no effect on downstream processes. Taq RedPOL is added directly to the reaction mix and is used in the same manner as standard Taq DNA Polymerase.

Taq RedPOL DNA Polymerase has both a 5'→3' DNA polymerase and a 5'→3' exonuclease activity. The enzyme lacks a 3'→5' exonuclease activity. Taq RedPOL DNA Polymerase leaves an A′ overhang, which makes the enzyme ideal for TA cloning.

Key Features

- High performance thermostable DNA polymerase
- Red dye identifies tubes which contain enzyme and confirms complete mixing of reagents
- Leaves an A′ overhang

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 Buffer

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

10X Ammonium Reaction Buffer

Tris-HCl pH 8.5, $(NH_4)_2SO_4$, 15 mM $MgCl_2$,
1% Tween 20

10X Standard Reaction Buffer

100mM Tris-HCl pH 8.3, 500mM KCl, 15mM $MgCl_2$,
1% Triton X-100.

Suggested Protocol using Taq RedPOL DNA Polymerase

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

Set up reaction mixtures in an area separate from that used for DNA preparation or product analysis.

1. Thaw 10X Buffer, dNTP mix, and 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.

The optimal $MgCl_2$ concentration should be determined empirically but in most cases a concentration of
1.5mM, as provided in the 1X Buffer, will produce satisfactory results. Table 2 provides the volume of 25mM $MgCl_2$ to add to the master mix if a higher $MgCl_2$ concentration is required.

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

Component	**Vol./reaction**	**Final Conc.**
10X Buffer	5 µL	1X
dNTP mix (12.5 mM of each)	0.8 µL	0.2 mM of each dNTP
Primer A	Variable	0.1–0.5 µM
Primer B	Variable	0.1–0.5 µM
Taq RedPOL DNA Polymerase	1 µL	5 units/reaction
Distilled Water	Variable	- - - -
Template DNA	Variable	0.1-0.5 µg/reaction
TOTAL volume	50 µL	- - - -

Table 2. $MgCl_2$ concentration

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 (0.1–0.5 µg/reaction) 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.

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

7. After primer extension, load 5-10 µL of a 50 µL reaction directly on an agarose gel for analysis.

Three-step PCR Programme

Cycles	Duration of cycle	Temperature
25-35	20 - 30 seconds[a]	95 °C
	20 – 40 seconds[b]	50-65 °C
	30 seconds[c]	72 °C
1	5 minutes[d]	72 °C

[a] Denaturation step: This step is the first regular cycling event and consists of heating the reaction to 95 °C for 20–30 seconds. It causes DNA melting of the DNA template by disrupting the hydrogen bonds between complementary bases, yielding single-stranded DNA molecules.

[b] Annealing step: The reaction temperature is lowered to 50–65 °C for 20–40 seconds allowing annealing of the primers to the single-stranded DNA template. Typically, the annealing temperature is about 3-5 degrees Celsius below the Tm of the primers used.

[c] Extension/elongation step: Taq polymerase has its optimum activity temperature at 72 °C. At this step the DNA polymerase synthesizes a new DNA strand complementary to the DNA template strand. The extension time depends on the length of the DNA fragment to be amplified. As a rule of thumb, at its optimum temperature the DNA polymerase will polymerize a thousand bases per minute.

[d] Final elongation: This single step is occasionally performed at a temperature of 72 °C for 5 minutes after the last PCR cycle to ensure that any remaining single-stranded DNA is fully extended.

Quality Control

Endonuclease, exonuclease and priming activities are not detected after 3 hours incubation of 1 µg of pUC19 plasmid DNA and 0.5 µg EcoR I digested lambda phage DNA at 72°C in the presence of 40 units of Taq RedPOL DNA Polymerase.

Related products

Description	Cat. no.
Taq DNA Polymerase (1000 units) Glycerol free	**100103**
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 r) with 2.0 mM $MgCl_2$	**150301**
Taq DNA Polymerase 2,0X MaMi RED (100 r) with 1.5 mM $MgCl_2$,	**180301**
Taq DNA Polymerase 2.0X MaMi RED (100 r) with 2.0 mM $MgCl_2$	**190301**
AccuPOL DNA Polymerase (500 units)	**210303**
TEMPase Hot Start DNA Polymerase (500 units) with 10X TEMPase Buffer I with 10X TEMPase Buffer II	**220303**
TEMPase Hot Start 2 x Master Mix with TEMPase Buffer I (100 r)	**230301**
TEMPase Hot Start 2 x Master Mix with TEMPase Buffer II (100 r)	**230701**
TEMPase Hot Start 2 x Master Mix Blue With TEMPase Buffer I (100 r)	**230301**
TEMPase Hot Start 2 x Master Mix Blue With TEMPase Buffer II (100 r)	**230701**
RealQ PCR 2 x Master Mix (200 reactions) for probe	**250407**
RealQ PCR 2 x Master Mix (200 reactions) With green dye	**250507**
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**

(Other product sizes available)

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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.

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