

Polymerases

Various DNA Polymerases

Taq DNA Polymerase/Red Taq DNA Polymerase:

Basic Protocol:

Component	Volume (µl)	Final Conc.
10x Reaction buffer	5	1x
50 mM MgCl ₂ Solution	1,5	1,5 mM
12,5 mM dNTP-Mix	1	250 µM
Primer A	x	0,2-1µM
Primer B	x	0,2-1µM
Template DNA	x	<1µg
Red Taq-DNA-Poly [1U/µl]	1-2,5	1-2,5U
Taq-DNA-Poly [5U/µl]	0,2-0,5	1-2,5U
Water	x	
Final volume	50	

Enzyme Storage Buffer

20 mM Tris-HCl pH 8.0 (at 25 °C), 30 mM KCl, 0.1 mM DTT, 0.5% Tween 20, 50 % (v/v) Glycerol.

10 x Reaction Buffer

670 mM Tris-HCl pH 8.8 (at 25 °C), 166 mM (NH₄)₂SO₄, 4.5 % Triton® X-100, 2 mg/ml Gelatin.

Mg²⁺ Solution

50 mM MgCl₂ (recommended final concentration: 1 – 4 mM).

Storage

Store Taq DNA Polymerase at -20 °C in a constant temperature freezer. If stored as recommended it will remain stable at least for 12 months.

Hot Start / Red Hot Start DNA Polymerase:

Basic Protocol:

Component	Volume (µl)	Final Conc.
10x Reaction buffer	5	1x
50 mM MgCl ₂ Solution	1,5	1,5 mM
12,5 mM dNTP-Mix	1	250 µM
Primer A	x	0,2-1µM
Primer B	x	0,2-1µM
Template DNA	x	<1µg
Hot Start DNA-Poly [5U/µl]	0,2-0,5	1-2,5U
Water	x	
Final volume	50	

10 x Reaction Buffer

100 mM Tris-HCl pH 8.3 (at 25 °C), 500 mM KCl, 0.1% Triton® X-100, Mg²⁺ Solution 50 mM MgCl₂ (recommended final concentration: 1 – 4 mM).

Storage

Store Hot Start DNA Polymerase at -20 °C in a constant temperature freezer. If stored as recommended it will remain stable at least for 12 months.

pfu DNA Polymerase/Long High fidelity Enzyme Mix:

Storage buffer

20mM Tris/HCl (pH 8.2 at 25C), 1mM DTT, 0.1mM EDTA, 100mM KCl, 0.1% (v/v) Tween 20, 50% (v/v) glycerol.

10x Reaction Buffer

500mM Tris/HCl, 140mM (NH₄)₂SO₄, 17.5mM MgCl₂, (pH 9.1 at 25C).

Unit Definition

One unit catalyzes the incorporation of 10nmol of deoxyribonucleotides into a polynucleotide fraction (adsorbed on DE-81) in 30min at 72C.

Template (temperature cycle)

Animal genomic DNA:	50-200 ng (25-35 cycles)
	10-50 ng (30-40 cycles)
Bacterial genomic DNA:	10-50 ng (20-25 cycles)
	1-5 ng (30-35 cycles)
Plasmid and lambda DNA:	1-5 ng (20-30 cycles)

DNA Polymerase

For amplification of longer fragments of an animal genomic DNA, the amount of enzyme should be increased to 2 or 2.5 U.

Extension time

The extension time should be 2 min/kb for Pfu Polymerase.

For High Fidelity Polymerase the extension time should be 30 sec/kb for short fragments and 1 min/kb for fragments longer than 5 kb.

For the amplification of fragments longer than 5 kb, the temperature ought to be assigned at 68C.