

Sequencing Pol

Taq Pol mutant for incorporation of ddNTPs

Thermus species, recombinant, E. coli

Cat.-No.	Size	Conc.
PCR-206S	200 units	5 units/ μ l
PCR-206L	1000 units	5 units/ μ l

For *in vitro* use only

Quality guaranteed for 12 months

Store at -20°C, avoid frequent thawing and freezing

Description

Sequencing Pol is a Taq polymerase mutant showing an enhanced efficiency for incorporation of ddNTPs. Its capability of incorporating ddNTPs and dNTPs at equal rates makes it the ideal choice for DNA cycle sequencing.

The enzyme replicates DNA at 74°C. It catalyzes the polymerization of nucleotides into duplex DNA in 5'→3' direction in the presence of magnesium and has a 5'→3' polymerisation-dependent exonuclease replacement activity only.

Unit definition

One unit is defined as the amount of enzyme required to catalyze the incorporation of 10 nmol of dNTP into an acid-insoluble form in 30 minutes at 74°C.

Recommended PCR assay

50 μ l PCR assay		
5 μ l	10x Sequencing buffer	blue cap
6 μ l	MgCl ₂ stock solution	yellow cap
200 μ M	each dNTP	
0.2-1 μ M	each Primer	
2-50 ng	Template DNA	
0.5-1 μ l (2.5-5 u)	Sequencing Pol	red cap
Fill up to 50 μ l	PCR grade H ₂ O	

Optimization of MgCl₂ concentration

A final Mg²⁺ concentration of 2.5-3.5 mM is recommended for most applications. Use the MgCl₂ stock solution for individual optimization as shown in the table below.

50 μ l PCR assay				
MgCl ₂ stock. (25 mM)	5 μ l	6 μ l	7 μ l	8 μ l
Final MgCl ₂ conc.	2.5 μ l	3 mM	3.5 mM	4 mM

Sequencing Pol (red cap)

5 units/ μ l Sequencing Pol in storage buffer

10x Sequencing buffer without MgCl₂ (blue cap)

500 mM Tris-HCl, pH 9.5 (25°C)

MgCl₂ stock solution (yellow cap)

25 mM MgCl₂