

## Taq DNA Polymerase with Robust Buffer (+ dNTPs)

| Product code          | 02-002 200 U 02-002-5 200   | U x 5  |  |
|-----------------------|---|--|--|
| Size                  | 200 U   |  |  |
| Storage               | -20°C   |  |  |
| Concentration         | 5 U/µl  |  |  |
| Product               | Thermus aquaticus DNA polymerase (Taq DNA polymerase) was expressed in E. coli in large   |  |  |
| Description           | quantities and highly purified. The enzyme has thermostable DNA polymerase activity and<br>the MW is 94 kDa. This enzyme is suitable for PCR reactions; capable of amplifying DNA |  |  |
|                       |   |  |  |
|                       | with various primers.   |  |  |
| Definition of         | One unit is defined as the amount of enzyme that can incorporate 10nmols of total dNTPs   |  |  |
| activity              | into an acid-insoluble material in 30 minutes at 74°C when activated salmon sperm DNA was   |  |  |
| •                     | used as template / primer.  |  |  |
| Purity                | Greater than 95% purity as determined by SDS-PAGE (CBB staining) (Fig.1)  |  |  |
|                       | The absence of endonucleases and e  | xonucleases was confirmed.   |  |
| PCR Test              | Good amplification result was obtained in PCR reaction using $\lambda DNA$ as a template up to 14   |  |  |
|                       | kB (Fig.2)  |  |  |
| Components            | Taq DNA polymerse (5U/µl): 20 mM Tris-HCl (pH 8.0), 100 mM KCl, 0.1 mM EDTA, 1 mM   |  |  |
|                       | DTT, 50% glycerol, 0.5% Tween20, 0.5% Igepal CA-630 (02-Taq 40µl)   |  |  |
|                       | 10 x Robust Buffer (Taq) (02-Trb 1.0ml)   |  |  |
|                       | <b>2.5 mM (each) dNTPs</b> (02-Dnt 640 µl)  |  |  |
| Application           | 1. High-throughput PCR  | General composition of PCR reaction mixture (total 50µl)   |  |
|                       | 2. Colony PCR   | Taq DNA polymerase (5 U/µl) (02-Taq)   |  |
|                       | 3. Incorporation of dUTP, dITP,   | $10x$ Robust Buffer (Taq) (02-Trb) $5 \ \mu l$ $2.5 \text{mM}$ (each) dNTPs (02-Dnt) $4 \ \mu l$ |  |
|                       | and fluorescence-labeled  | Template <500 ng<br>Primer 1 0.2~1.0 mM (final conc.)  |  |
|                       | nucleotides   | Primer 2 0.2~1.0 mM (final conc.)  |  |
|                       | 4. Primer extension   | Sterile distilled water up to 50 µl  |  |
|                       | 5. Addition of a single nucleotide<br>(adenosine) at the 3'-blunt ends  | X Use of excess amount is not recommended.   |  |
|                       | for cloning into TA vector.   |  |  |
|                       | Cautions for using Robust Buffer ( <i>Taq</i> )   |  |  |
|                       | Robust Buffer induces maximum enzymatic activity. To avoid production of undesirable  |  |  |
|                       | smear bands in gel electrophoresis analysis, the optimal reaction time is recommended as  |  |  |
|                       | follows: 1) about 5 to 10 seconds / kb elongation time for template up to 8 kb, and about 15  |  |  |
|                       | seconds / kb for up to 14 kb; 2) roughly the same elongation time is set with 2-step PCR (shuttle   |  |  |
|                       | PCR) and 3-step PCR; 3) extend the  | elongation time by short steps when amplification is not   |  |
|                       | seen. Amplification can be detected   | more rapidly by adopting 2-step PCR.   |  |
| Related product       | 02-001 Taq DNA Polymerase with Standard Buffer (+dNTPs)   |  |  |
|                       | 02-021 Pfu DNA Polymerase with Standard Buffer (+dNTPs)   |  |  |
| Please note: All proc | ducts are FOR RESEARCH USE ONL  | Y. NOT FOR USE IN DIAGNOSTIC   |  |
| PROCEDURES. NO        | OT FOR MILITARY USE.  |  |  |
|                       |   |  |  |



Data Images: 02-002 Taq DNA Polymerase with Robust Buffer (+dNTPs)

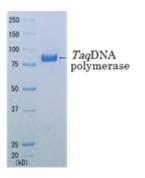


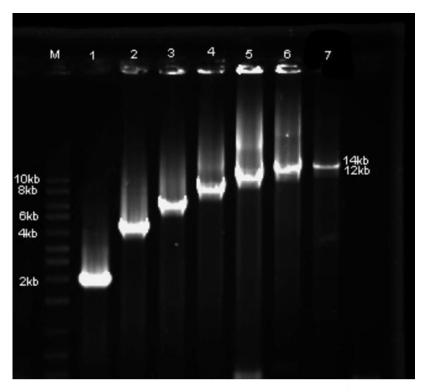
Fig.1 SDS-PAGE of TaqDNA polymerase

## Protocols for PCR

Examples of PCR conditions for the amplification of various sizes of  $\lambda$ DNA (results shown in Fig.2)

## 2 kb, 4 kb

| 94 °C<br>95 °C<br>65 °C          | $ \begin{array}{c} 1 \text{ min} \\ 5 \text{ sec} \\ 20 \text{ sec} \end{array} \right\} 25 \text{ cycles} $  |  |  |
|----------------------------------|---|--|--|
| 6 kb                             |   |  |  |
| 94 °C<br>95 °C<br>65 °C          | $\begin{array}{c} 1 \text{ min} \\ 5 \text{ sec} \\ 1 \text{ min} \end{array} \right\} 25 \text{ cycles}$   |  |  |
| 8 kb                             |   |  |  |
| 94 °C<br>95 °C<br>65 °C          | $ \begin{array}{c} 1 & \min \\ 5 & \sec \\ 1 & \min 20 & \sec \end{array} \right\} 25 \text{ cycles} $  |  |  |
| 10 kb, 12 kb                     |   |  |  |
| 94 °C                            | 1 min   |  |  |
| 14 kb                            |   |  |  |
| 94 °C<br>98 °C<br>68 °C<br>72 °C | $ \begin{array}{c} 1 \text{ min} \\ 5 \text{ sec} \\ 4 \text{ min} \\ 4 \text{ min} \end{array} $ $ \begin{array}{c} 30 \text{ cycles} \\ 4 \text{ min} \end{array} $ |  |  |



M: marker, lane1: 2kb, lane2: 4kb, lane3: 6kb, lane4: 8kb, lane5: 10kb, lane6: 12kb, lane7: 14kb,

## Fig.2 PCR products obtained by using Robust buffer (agarose gel electrophoresis)