

# TaKaRa Ex Taq™

Code No. HRR001A  
Size: 250 units

Shipping at - 20°C  
Store at - 20°C

Supplied Reagents: 10X Ex Taq Buffer 1 ml  
dNTP Mixture 800 µl

Lot No.  
Conc.: units/µl  
Volume: µl  
Expiration Date:

Storage Buffer: 20 mM Tris-HCl (pH8.0)  
100 mM KCl  
0.1 mM EDTA  
1 mM DTT  
0.5% Tween 20  
0.5% NP-40  
50% Glycerol

Supplied 10X Ex Taq Buffer: Mg<sup>2+</sup> concentration (10X): 20 mM

#### Supplied dNTP Mixture:

dNTP Mixture is ready for use in PCR without dilution.  
Concentration : 2.5 mM of each dNTP  
Form : Dissolved in water (sodium salts), pH 7 - 9  
Purity : ≥ 98% for each dNTP

**Unit definition:** One unit is the amount of enzyme that will incorporate 10 nmol of dNTP into acid-insoluble products in 30 minutes at 74°C with activated salmon sperm DNA as the template-primer.

#### Reaction mixture for unit definition:

25 mM TAPS (pH 9.3 at 25°C)  
50 mM KCl  
2 mM MgCl<sub>2</sub>  
0.1 mM DTT  
200 µM each dATP·dGTP·dCTP  
100 µM [<sup>3</sup>H]-dTTP  
0.25 mg/ml activated salmon sperm DNA

**Purity:** Nicking, endonuclease and exonuclease activity were not detected after the incubation of 0.6 µg of supercoiled pBR322 DNA, 0.6 µg of λ DNA or 0.6 µg of λ-Hind III digest with 10 units of this enzyme for 1 hour at 74°C.

**Applications:** For DNA amplification by Polymerase Chain Reaction (PCR).

**PCR products:** As most PCR products amplified with TaKaRa Ex Taq™ have one A added at 3'-termini, the obtained PCR product can be directly used for cloning into T-vector. Also it is possible to clone the product in blunt-end vectors after blunting and phosphorylation of the end.

**PCR test:** Good performance of DNA amplification by PCR was confirmed by using λ DNA as the template (amplified fragment: 20 kb). Good performance of DNA amplification of β-globin gene by PCR was also confirmed by using human genomic DNA as the template (amplified fragment: 17.5 kb).

#### General reaction mixture for PCR (total 50 µl)

TaKaRa Ex Taq™ (5 units/µl) 0.25 µl  
10X Ex Taq Buffer 5 µl  
dNTP Mixture (2.5 mM each) 4 µl  
Template < 500 ng  
Primer 1 0.2 - 1.0 µM (final conc.)  
Primer 2 0.2 - 1.0 µM (final conc.)  
Sterilized distilled water up to 50 µl

#### PCR condition (an example):

When amplifying 1 kb DNA fragment  
98°C 10 sec. } 30 cycles or 98°C 10 sec. } 30 cycles  
55°C 30 sec. }  
72°C 1 min. }

(Note) Denaturation conditions vary depending on the thermal cycler and tubes used for PCR. The recommendation is for 1 - 10 sec. at 98°C or 10 - 30 sec. at 94°C.

#### < Cool Start Method >

"Cool Start Method" + provides more accurate amplification and minimizes amplification of nonspecific bands. This is a simple method that does not require specialized enzymes or additional reagents. Higher reaction specificity can be achieved by combining Hot Start PCR techniques with Taq Antibody (Cat. #9002A) and Cool Start method.

#### Protocol of Cool Start Method

- 1) Keep all reagents on ice until use.
  - 2) Prepare the reaction mixture on ice.\*1.\*2  
\*1: Order of reagent addition does not influence results.  
\*2: Results will not be affected by leaving the mixture on ice for 30 min. before thermal cycling.
  - 3) Set a thermal cycler with the designated program.\*3  
\*3: PCR conditions do not need to be changed for Cool Start.
  - 4) Set the tubes in a thermal cycler and start thermal cycling immediately.
- + : JAPAN Patent 2576741 for Cool Start Method is owned by SHIMADZU CORPORATION

#### NOTICE TO PURCHASER: LIMITED LICENSE

##### [P1] PCR Notice

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##### [M57] LA Technology

This product is covered by the claims 6-16 of U.S. Patent No. 5,436,149 and its foreign counterpart patent claims.

#### Note

This product is for research use only. It is not intended for use in therapeutic or diagnostic procedures for humans or animals. Also, do not use this product as food, cosmetic, or household item, etc. Takara products may not be resold or transferred, modified for resale or transfer, or used to manufacture commercial products without written approval from TAKARA BIO INC. If you require licenses for other use, please contact us by phone at +81 77 543 7247 or from our website at www.takara-bio.com.