2XPCR Solution

Premix Ex Taq™ Hot Start Version

Code No. RR030A
Size: 500 μl X 5
(for 100 PCR reactions)

Shipping at -20°C
Stored at -20°C

Storage: Repeated freeze-thaw cycles may decrease the enzyme activity. Once it thawed, dispense into PCR tubes and store at -20°C. (ex. For 50 μl PCR reaction, dispense by 25 μl each tube.)

Lot No.

Expiry Date :

Description:
This product is an optimized mixture composed of enzyme (*TaKaRa Ex Taq*™ HS), reaction buffer and dNTP mixture as 2-fold concentrations.
TaKaRa Ex Taq™ HS is designed to be suitable for Hot Start PCR. It is derived from *TaKaRa Ex Taq*™ and neutralizing monoclonal antibody to *Taq* DNA polymerase. Non-specific amplification due to mispriming and/or formation of primer dimer before thermal cycling can be prevented, since the antibody inhibits the polymerase activity by binding to the *Taq* DNA polymerase until the temperature elevates. This enzyme can be used in general PCR conditions, since monoclonal antibody is denatured in the initial DNA-denaturation step.

Content:

TaKaRa Ex Taq™ HS *:	1.25 units/ 25 μl
dNTP Mixture	: 2X conc.; ea. 0.4 mM
Ex Taq™ buffer	: 2X conc.; including 4 mM Mg^{2+}

***Specification of *TaKaRa Ex Taq*™HS (TaKaRa Cat.#RR006)**

Unit definition: One unit is the amount of the enzyme that will incorporate10 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	$MgCl_2$
1 mM	2-mercaptoethanol
200 μM	each dATP,dGTP,dTTP
100 μM	[α-^{32}P]-dCTP
0.25 mg/ml	activated salmon sperm DNA

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

Test for antibody: Inhibition of *Ex Taq* activity by the antibody is confirmed to be more than 90% after the reaction at 55°C for 10 min.

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

PCR product: As most PCR products amplified with *TaKaRa Ex Taq*™ HS 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 λDNA as the template (amplified fragment : 20 kbp). Good performance of DNA amplification of a single copy gene by PCR was also confirmed by using human genome DNA (amplified fragment : 2.9 kbp).

General reaction mixture for PCR (total 50 μl)

Premix Ex Taq™ Hot Start Version*	25 μl
Template	<1 μg
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

* Please mix gently to be uniform and then use.

PCR conditions
This enzyme can be used in general PCR conditions, since the monoclonal antibody is denatured in the initial DNA-denaturation step. No need for a special step to denature the antibody to *Taq* polymerase.

(Example) Amplification of 1 kbp DNA fragment

98°C 10 sec.
55°C 30 sec.
72°C 1 min.
] 30 cycles

or

98°C 10 sec.
68°C 1 min.
] 30 cycles

Note:
Denaturation condition varies depending on an used thermal cycler and tube. It is recommended for 20 - 30 sec. at 94°C, or 5 - 10 sec. at 98°C.

Note
For research use only. Not for use in diagnostic or therapeutic procedures.

U.S. Patent 5,436,149 for LA Technology is owned by TAKARA BIO INC.

NOTICE TO PURCHASER: LIMITED LICENSE

A license under the foreign counterparts of U.S. Patents Nos. 4,683,202, 4,683,195 and 4,965,188 owned by F.Hoffmann-La Roche Ltd. and U.S. Patent No. 5,075,216 and its foreign counterpart, owned by Roche Molecular Systems, Inc. and F.Hoffmann-La Roche Ltd. for use in research and development, has an up-front fee component and a running-royalty component. The purchase price of this product includes limited, nontransferable rights under the running-royalty component to use only this amount of the product to practice the polymerase chain reaction (PCR) and related processes described in said patents where such processes are covered by patents solely for the research and development activities of the purchaser when this product is used in conjunction with a thermal cycler whose use is covered by the up-front fee component. Rights to the up-front fee component must be obtained by the end user in order to have a complete license to use this product in the PCR process where the process is covered by patents. These rights under the up-front fee component may be purchased from Applied Biosystems or obtained by purchasing an authorized thermal cycler. No right to perform or offer commercial services of any kind using PCR, where the process is covered by patents, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is hereby granted by implication or estoppel. Further information on purchasing licenses to practice the PCR process where the process is covered by patents may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404 or the Licensing Department, Roche Molecular Systems, Inc., 1145 Atlantic Avenue, Alameda, California 94501.

Produced by TAKARA BIOTECHNOLOGY (DALIAN) CO.,LTD. v2006.04Da

2×PCR Solution

Premix Ex Taq™ Hot Start Version

Code No. RR030A
Size: 500 μl X 5
(for 100 PCR reactions)

Shipping at -20℃
Stored at -20℃

保存 : 凍結融解の繰り返しにより活性が低下する恐れがあります。融解後は、ＰＣＲ用のチューブに小分けして、－20℃保存してください。(50 μl反応の場合、25 μlずつ)

Lot No. (英文面をご覧ください。)

品質保証期限 : (英文面をご覧ください。)

●製品説明

本製品は、*TaKaRa Ex Taq*® HS、反応バッファー、dNTP Mixtureをあらかじめ2倍濃度で混合したものである。*TaKaRa Ex Taq*® HSは、抗*Taq*抗体と*TaKaRa Ex Taq*®を混合したホットスタートPCR用の酵素で、高温に加熱するまでは抗*Taq*抗体が酵素に結合しポリメラーゼ活性を抑えているため、サイクル前のミスプライミングやプライマーダイマーに由来する非特異的増幅を防ぐことができる。抗*Taq*抗体は、PCRの最初のDNA変性ステップで変性するため、従来のPCR条件で反応できる。抗*Taq*抗体を失活させるための特別なステップは必要ない。

●内容

TaKaRa ExTaq® HS *	: 1.25 units/ 25 μl
dNTP Mixture	: 2Xconc.; 各0.4 mM
Ex Taq™ buffer	: 2Xconc.; 4 mM Mg^{2+}を含む

●* *TaKaRa Ex Taq*® HS (Code RR006)

○活性の定義

活性化サケ精子ＤＮＡを鋳型／プライマーとして用い、下記の活性測 定用反応液中にて74℃において、30分間に10 nmolの全ヌクレオチドを酸不溶性沈殿物に取り込む活性を1Uとする。

○活性測定用反応液組成

25 mM	TAPS緩衝液（pH9.3、25℃）
50 mM	KCl
2 mM	$MgCl_2$
1 mM	2-メルカプトエタノール
各200 μM	dATP･dGTP･dTTP
100 μM	［α-^{32}P］dCTP
0.25 mg/ml	活性化サケ精子DNA

○純度

1. **10Uの本酵素と0.6 μgのλ-*Hin*d III分解物とを74℃、 1時間反応させてもDNAの電気泳動パターンに変化は起こらない。**
2. **10Uの本酵素と0.6 μgのsupercoiled pBR322 DNAとを74℃、1時間反応させてもDNAの電気泳動パターンに変化は起こらない。**
3. **10Uの本酵素と0.6μgのλ ＤＮＡとを74℃、1時間反応させてもDNAの電気 泳動パターンに変化は起こらない。**

○検定

55℃、10分間の反応での抗体による*Ex Taq*活性の阻害率が90%以上であることを確認している。

●用途

Polymerase Chain Reaction (PCR)法によるDNA増幅

●PCR産物

***TaKaRa Ex Taq*® HSを用いて増幅したPCR産物のほとんどは、3'末端にAが1塩基付加されている。したがって、そのＰＣＲ産物をそのままT-vectorにクローニングすることが可能である。また、末端平滑化およびリン酸化を行って、平滑末端のベクターにクローニングすることも可能である。**

●PCR検定

1. **λ DNAを鋳型としたPCR反応（増幅産物20 kbp）において良好な増幅が見ら れることを確認している。**
2. **ヒトゲノムDNAを鋳型としたsingle copy geneのPCR反応（増幅産物2.9 kbp）において良好な増幅が見られることを確認している。**

●PCR反応例 (total 50 μl PCR)

Premix Ex Taq™ Hot Start Version*	25 μl
Template	<1 μg
Primer 1	0.2 ～ 1.0 μM (final conc.)
Primer 2	0.2 ～ 1.0 μM (final conc.)
滅菌蒸留水	up to 50 μl

*** 均一になるまでゆるやかに転倒混合して使用する。**

●PCR条件

PCRの最初のDNA変性ステップで抗*Taq*抗体は失活するので、従来のPCR条件が使用できる。抗*Taq*抗体を失活させるための特別なステップは必要ない。

（例） 1 kbp DNA**を増幅する時**

98**℃** 10 sec.
55**℃** 30 sec.
72**℃** 1 min.
] 30 cycles or
98**℃** 10 sec.
68**℃** 1 min.
] 30 cycles

注）変性の条件は、サーマルサイクラーの使用機種と反応チューブの種類に合わせて設定してください。設定の目安としては、94℃の場合は20～30 sec.、98℃の場合は5～10 sec.です。

●注意

本製品は研究用試薬です。ヒト、動物への医療、臨床診断には使用しないようご注意ください。また、食品、化粧品、家庭用品等として使用しないでください。

製品についての技術的なお問い合わせ先 **TaKaRaテクニカルサポートライン** Tel 077-543-6116 Fax 077-543-1977