Cat. # R076A

For Research Use

TaKaRa

# MightyAmp™ DNA Polymerase Ver.3

Product Manual

v201805Da

# Table of Contents

## I. Description

MightyAmp DNA Polymerase is a PCR enzyme developed for ultimate reactivity. Because of its strong amplification ability, it performs well even with samples that would otherwise cause amplification difficulties, such as crude biological extracts containing high amounts of PCR-inhibiting substances.
MightyAmp DNA Polymerase Ver.3 combines MightyAmp DNA Polymerase with an improved PCR enzyme and buffer, further increasing its resistance to PCR-inhibiting substances when compared to Ver.2. In addition, its performance in direct PCR (i.e., directly adding a biological sample such as blood, animal/plant tissue, etc. into a PCR reaction) is also improved.
This enzyme allows the amplification of a wide range of targets from many types of samples, regardless of GC or AT content, or the amount of PCR inhibiting substances in crude samples. Furthermore, it is possible to increase specificity and sensitivity of PCR amplification by adding the provided 10X Additive for High Specificity to a PCR reaction mixture, as necessary.
This enzyme is appropriate for use in hot-start PCR, which inhibits polymerase activity up to 98℃.

## II. Components (50 μl reactions, for 200 total reactions)

| | |
|---|---|
| MightyAmp DNA Polymerase Ver.3 (1.25 U/μl) | 200 μl |
| 2X MightyAmp Buffer Ver.3 ($Mg^{2+}$, dNTP plus) * | 1 ml x 5 |
| 10X Additive for High Specificity | 500 μl x 2 |

* 4 mM $Mg^{2+}$; 600 μM each dNTP

## III. Storage

-20℃

## IV. General PCR Reaction Mix

| Reagents | Amount | Final conc. |
|---|---|---|
| 2X MightyAmp Buffer Ver.3 | 25 μl | 1X |
| Primer 1 | 15 pmol | 0.3 μM |
| Primer 2 | 15 pmol | 0.3 μM |
| Sample/crude extract*1 | Appropriate | |
| MightyAmp DNA Polymerase Ver.3 | 1 μl | 1.25 U/50 μl |
| (10X Additive for High Specificity | 5 μl | 1X)*2 |
| Sterile purified water | to 50 μl | |
| Total | 50 μl | |

*1 Recommended amount used for each tissue and extract
- Blood treated with EDTA or heparin ≤10 μl
- Blood treated with citrate*3 ≤5 μl
- Mouse tail ≤1 mm
- Mouse ear punch ≤1.5 $mm^2$
- Mouse organ or brain ≤1.5 $mm^3$
- Plant leaf (tomato, *Arabidopsis*, spinach), ≤2 mm diameter
- Crude extract from a biological sample ≤5 μl

*3 The reactivity may be decreased with blood treated with citrate.

*2 First perform the reaction without the addition of 10X Additive for High Specificity. To increase amplification specificity or sensitivity, add 10X Additive for High Specificity.

## V. Primer Design

Select the best primer sequences using primer design software (e.g., OLIGO Primer Analysis Software: Molecular Biology Insights, Inc., etc.).

We recommend primers with a Tm of 60℃ or more, as calculated by the equation below.

Tm (℃) = [(the number of A and T) x 2] + [(the number of G and C) x 4] + 35 - 2 x [total bases]

## VI. PCR Conditions

For standard conditions, use a 3-step PCR protocol with an extension temperature of 68℃. When amplifying a GC-rich target, use a 2-step PCR protocol.

[3-step PCR]

| | | |
|---|---|---|
| 98℃ | 2 min*1 | |
| ↓ | | |
| 98℃ | 10 sec | ⎫ |
| 60℃ | 15 sec | ⎬ 30 - 40 cycles |
| 68℃*2 | 1 min/kb | ⎭ |

[2-step PCR]

| | | |
|---|---|---|
| 98℃ | 2 min*1 | |
| ↓ | | |
| 98℃ | 10 sec | ⎫ |
| 68℃ | 1 min/kb | ⎭ 30 - 40 cycles |

*1 Because a robust hot-start antibody is included, an initial thermal denaturation step (98℃, 2 min) should be included in the PCR program to denature the antibody.

*2 Set the extension temperature to 68℃ for 3-step PCR.

## VII. Analysis of Amplified Products

- We recommend using TAE buffer for gel electrophoresis of PCR products amplified with MightyAmp DNA Polymerase Ver.3. If you use TBE buffer, you may not get clear results due to electrophoretic pattern widening towards the bottom of the gel.
- For gel electrophoresis of PCR products amplified directly from animal tissue (e.g., mouse tail), the DNA fragment may get trapped in the well of the agarose gel and may not migrate properly due to insoluble matter (tissue) remaining in the sample solution. In this instance, we recommend adding Proteinase K to the Loading Buffer as follows.
  1. Mix 6X Loading Buffer (Cat. #9156) and Proteinase K (Cat. #9034) in a 10 : 1 (v/v) ratio.
  2. Add the mixture from in Step 1 to the PCR reaction in a 1 : 5 (v/v) ratio before performing gel electrophoresis.

## VIII. Cloning PCR Products

Most of the PCR products amplified with MightyAmp DNA Polymerase Ver.3 have a one-base (A) addition at their 3′ -terminal end. Therefore, you can clone PCR products directly into a T-Vector (pMD20, Cat. #3270; pMD19 (Simple), Cat. #3271, etc.). It is also possible to clone into a vector with blunt ends after blunting and phosphorylation with the Mighty Cloning Reagent Set (Blunt End) (Cat. #6027).

## IX. Troubleshooting

| Problem | Cause | Solution |
|---|---|---|
| No amplification<br>Low amplification efficiency | Primer Tm value | Design primers as described in Section IV. |
| | 2-step PCR | Try 3-step PCR. |
| | 3-step PCR | Try 2-step PCR (sometimes a target not amplified by 3-step PCR can be amplified by 2-step PCR. |
| | Annealing temperature | Progressively lower temperature in 2℃ increments. |
| | Cycle number | Increase cycle number (up to a maximum of 40 cycles). |
| | Amount of sample or sample preparation | • Reduce or increase amount of sample used.<br>• Examine sample preparation method. |
| | Reaction mixture composition | Add the 10X Additive for High Specificity when performing PCR.* |
| Extreme non-specific amplification | Primer Tm value | Design primers as described in Section IV. |
| | 3-step PCR | Try 2-step PCR. |
| | Cycle number | Set cycle number to 25 - 30 cycles. |
| | Sample preparation | Examine the sample preparation method. |
| | Reaction mixture composition | Add the 10X Additive for High Specificity when performing PCR.* |

* For more information on increasing amplification specificity and sensitivity with the 10X Additive for High Specificity, refer to Section X. Experimental Examples are on the next page.

## X. Experimental Examples

### X-1. PCR with high humic acid concentration

Humic acid present in soil is known to strongly inhibit PCR reactions. MightyAmp DNA Polymerase Ver.3 has a remarkably high resistance to humic acid when compared to other PCR enzymes. This feature allows MightyAmp DNA Polymerase Ver.3 to amplify PCR products from crude samples containing high levels of soil components.

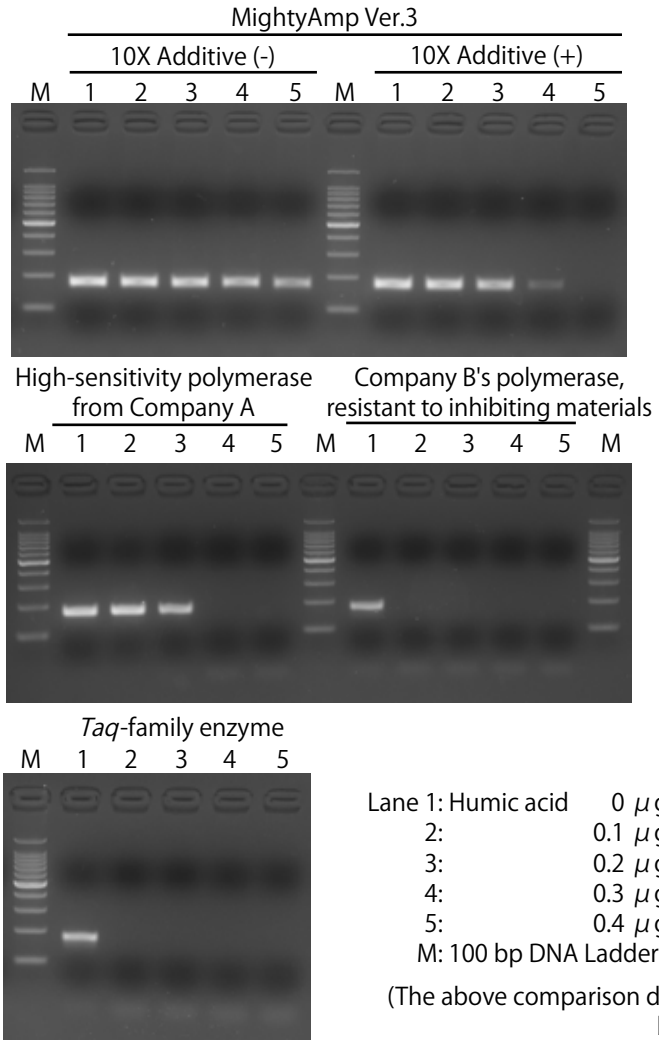
Template: *E. coli* genomic DNA (equivalent to 2 x $10^5$ copies)

Target: 16S rDNA (173 bp)

Reaction conditions: as recommended for each enzyme

PCR conditions for MightyAmp DNA Polymerase Ver.3:

| | | |
|---|---|---|
| 98℃ | 2 min | |
| ↓ | | |
| 98℃ | 10 sec | 30 cycles |
| 60℃ | 15 sec | |
| 68℃ | 30 sec | |




Lane 1: Humic acid 0 μg
2: 0.1 μg
3: 0.2 μg
4: 0.3 μg
5: 0.4 μg/25 μl reaction
M: 100 bp DNA Ladder (Cat. #3407A)

(The above comparison data was obtained by our company.)

### X-2. PCR with high NaCl concentration

Samples containing a high concentration of salt, such as seawater (containing about 500 mM NaCl), are known to inhibit PCR reactions.
This polymerase has a remarkably high resistance to salt concentration compared to other PCR enzymes, allowing PCR product amplification from crude samples with high concentrations of salt.

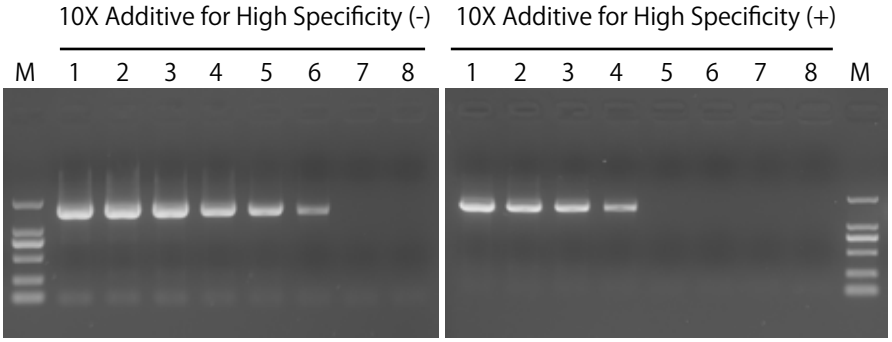
Template: *E. coli* genomic DNA (equivalent to 2 x $10^5$ copies)

Target: Total length of 16S rDNA (1,465 bp)

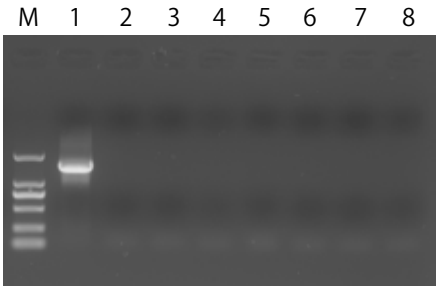
Reaction conditions: as recommended for each enzyme

PCR reaction conditions for MightyAmp DNA Polymerase Ver.3:

| | | |
|---|---|---|
| 98℃ | 2 min | |
| ↓ | | |
| 98℃ | 10 sec | ┐ |
| 60℃ | 15 sec | ├ 30 cycles |
| 68℃ | 90 sec | ┘ |




*Taq*-family enzyme




| | |
|---|---|
| Lane 1: Final NaCl concentration | 0 mM |
| 2: | 50 mM |
| 3: | 75 mM |
| 4: | 100 mM |
| 5: | 125 mM |
| 6: | 150 mM |
| 7: | 175 mM |
| 8: | 200 mM |

M: DL 2,000 DNA Marker (Cat. #3427A)

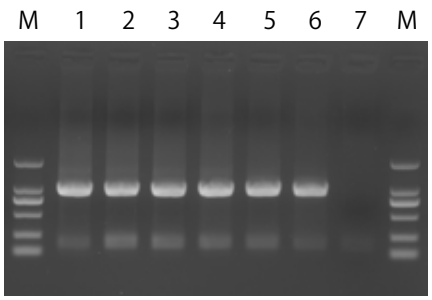
### X-3. Improved resistance to an inhibiting substance (e.g., tannic acid) by adding the 10X Additive for High Specificity

Tannic acid in plants is known to strongly inhibit PCR reactions.
This polymerase has a remarkably high resistance to tannic acid compared to other PCR enzymes, allowing successful amplification of PCR products from crude samples containing high amounts of plant compounds.

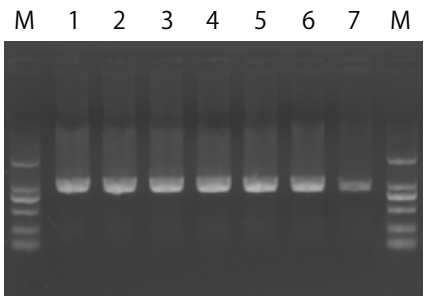
Template: Human genomic DNA (100 ng)
Target: Human *DCLRE1A* gene (1 kb)
Reaction conditions: recommended for each enzyme
PCR conditions for MightyAmp DNA Polymerase Ver.3:

| | | |
|---|---|---|
| 98℃ | 2 min | |
| ↓ | | |
| 98℃ | 10 sec | 30 cycles |
| 60℃ | 15 sec | |
| 68℃ | 1 min | |

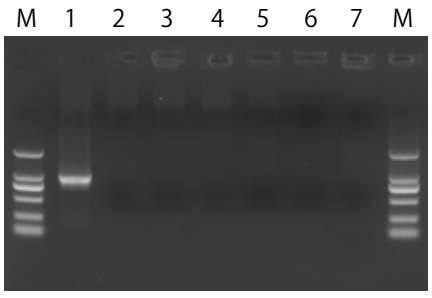
10X Additive for High Specificity (-)




10X Additive for High Specificity (+)




*Taq*-family enzyme




Lane 1: Tannic acid 0 ng/μl
2: 1 ng/μl
3: 5 ng/μl
4: 10 ng/μl
5: 50 ng/μl
6: 100 ng/μl
7: 250 ng/μl
M: DL 2,000 DNA Marker (Cat. #3427A)

### X-4. Increased PCR specificity by addition of 10X Additive for High Specificity

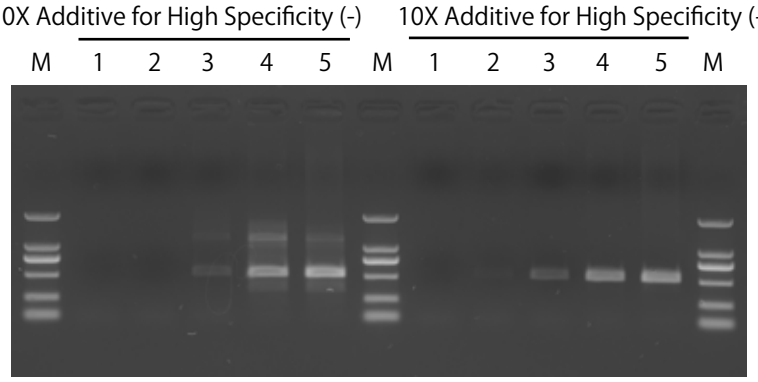
Template: Human genomic DNA

Target: *APOE* gene (520 bp)

10X Additive for High Specificity: without (-); with (+)

PCR conditions:

| | | | |
|---|---|---|---|
| 98℃ | 2 min | | |
| ↓ | | | |
| 98℃ | 10 sec | ┐ | |
| 60℃ | 15 sec | ├ | 30 cycles |
| 68℃ | 30 sec | ┘ | |




Lane 1: Human genomic DNA 100 pg
2: 1 ng
3: 10 ng
4: 100 ng
5: 500 ng/50 μl reaction
M: DL 2,000 DNA Marker (Cat. #3427A)

Note: We recommend using the 10X Additive for High Specificity in reactions using higher purity templates, such as purified genomic DNA.

## X-5. Direct PCR using various biological samples

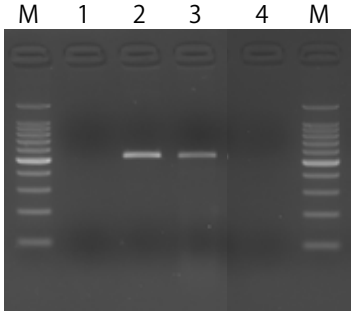
### (1) Direct PCR with mouse blood spotted on an FTA card

[Method] An FTA card spotted with mouse blood was cut with a 1.2-mm diameter punch and added to a 25-μl reaction mixture containing this product. Mouse *Hbb-b1* gene (542 bp) was amplified (3-step protocol, 30 cycles) using conditions recommended for each enzyme. Five microliters of each reaction was used for gel electrophoresis.

[Result] The target gene was well amplified from mouse blood spotted on an FTA card by direct PCR with MightyAmp DNA Polymerase Ver.3.

PCR conditions for MightyAmp DNA Polymerase Ver.3:

| | | |
|---|---|---|
| 98℃ | 2 min | |
| ↓ | | |
| 98℃ | 10 sec | 30 cycles |
| 60℃ | 15 sec | |
| 68℃ | 30 sec | |




Lane 1: MightyAmp Ver.2 (Cat. #R071A)*
2: MightyAmp Ver.3
10X Additive for High Specificity (-)
3: MightyAmp Ver.3
10X Additive for High Specificity (+)
4: Company B's polymerase, resistant to inhibiting materials
M: 100 bp DNA Ladder (Cat. #3407A)

(The above comparison data was obtained by our company.)

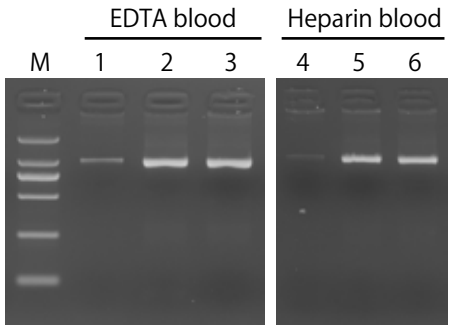
### (2) Direct PCR using human EDTA blood and heparin blood

[Method] Five microliters of human blood, treated with EDTA or heparin, was added to a 25-μl reaction mixture containing this product. A portion of the human *DCLRE1A* gene (~1 kb) was amplified (3-step protocol, 30 cycles). Five microliters of each reaction was used for gel electrophoresis.

[Result] The target gene was efficiently amplified from blood treated with EDTA or heparin by direct PCR with MightyAmp DNA Polymerase Ver.3.

PCR conditions:

| | | |
|---|---|---|
| 98℃ | 2 min | |
| ↓ | | |
| 98℃ | 10 sec | 30 cycles |
| 60℃ | 15 sec | |
| 68℃ | 1 min | |




Lane 1, 4: MightyAmp Ver.2 (Cat. #R071A)*
2, 5: MightyAmp Ver.3
10X Additive for High Specificity (-)
3, 6: MightyAmp Ver.3
10X Additive for High Specificity (+)
M: DL 2,000 DNA Marker (Cat. #3427A)

* Not available in all geographic locations. Check for availability in your area.

## XI. Related Products

MightyAmp™ DNA Polymerase Ver.2 (Cat. #R071A/B)*
MightyAmp™ Genotyping Kit (Cat. #R074A)*
Tks Gflex™ DNA Polymerase (Cat. #R060A/B)*
TaKaRa PCR Thermal Cycler Dice™ *Touch* (Cat. #TP350)*
TaKaRa PCR Thermal Cycler Dice™ Gradient (Cat. #TP600)
Agarose L03「TAKARA」(Cat. #5003/B)
T-Vector pMD20 (Cat. #3270)
T-Vector pMD19 (Simple) (Cat. #3271)
Lysis Buffer for PCR (Cat. #9170A)*
Plant DNA Isolation Reagent (Cat. #9194)
Proteinase K (Cat. #9034)
6X Loading Buffer (Cat. #9156)

* Not available in all geographic locations. Check for availability in your area.