

Cat. # 9182

For Research Use

---

**TAKARA**

**MightyPrep reagent for DNA**

---

Product Manual

v201509Da

---

## Table of Contents

I. Description.....	3
II. Components.....	3
III. Materials Required but not Provided .....	3
IV. Storage .....	3
V. Notes .....	3
VI. Protocol .....	3
VII. Experimental Examples.....	4
VIII. Related Products.....	6

## I. Description

MightyPrep reagent for DNA is designed to easily prepare DNA extracts that can be used directly as PCR templates—from animal tissues (such as mouse tail), plant tissues, blood, processed food, and soil. DNA extracts are prepared using a simple protocol that consists of adding the reagent to a sample, then incubating and centrifuging the mixture. These extracts can be used directly as PCR templates with inhibitor-resistant PCR enzymes such as MightyAmp® DNA Polymerase Ver.3 (Cat. #R076A/B)\* or Tks Gflex™ DNA Polymerase (Cat. #R060A/B)\* to successfully amplify targets. This product can also be used to efficiently prepare DNA extracts from microorganisms. The resulting extracts can be used as templates with the Bacterial 16S rDNA PCR Kit Fast (800) (Cat. #RR182A)\* or the Fungal rDNA (ITS1) PCR Kit Fast (Cat. #RR183A)\*.

\*: Not available in all geographical locations. Check for availability in your region.

## II. Components

MightyPrep reagent for DNA                      10 ml x 2

## III. Materials Required but not Provided

- Heat block (95°C)
- High-speed centrifuge
- Vortex mixer

## IV. Storage    4°C

## V. Notes

Some DNA extracts may contain contaminants that can cause PCR inhibition. In such cases, decrease the amount of DNA extract in the PCR reaction mixture by using diluted DNA extract. In addition, inhibitor-resistant PCR enzymes such as MightyAmp DNA Polymerase Ver.3 (Cat. #R076A/B)\* or Tks Gflex DNA Polymerase (Cat. #R060A/B)\* are recommended for increasing the success rate of PCR amplification. If PCR inhibition still occurs under such reaction conditions, purify the template DNA from the original samples using a DNA purification kit such as NucleoSpin Tissue (Cat. #740952.50/.250).

\* Not available in all geographical locations. Check for availability in your region.

## VI. Protocol

### VI-1. Standard protocol

1. Add samples\*<sup>1</sup> to 1.5-ml microcentrifuge tubes.
2. Add 100  $\mu$ l of MightyPrep reagent for DNA and vortex to mix.
3. Heat at 95°C for 10 min.
4. Centrifuge at 12,000 - 15,000 rpm for 2 min.
5. Use the supernatant as template for PCR.\*<sup>2,3</sup>

\* 1: Recommended amounts of starting samples: Blood: 2 - 20  $\mu$ l, mouse tail: 1 - 2 mm, plant tissue: 1 - 10 mm<sup>2</sup>, soil ~0.5 mg, etc.

\* 2: Use the extract as soon as possible. If you want to store it, transfer the supernatant to a new tube, and store at 4 °C.

\* 3: The amount of extract used for PCR should be less than 1/10 of the PCR reaction volume.

**VI-2. Protocol for bacterial samples**

- From a colony
  - (1) Add 100  $\mu$ l of MightyPrep reagent for DNA to a 1.5-ml tube.
  - (2) Use a sterile pipette tip or a loop to transfer a small amount of the bacterial colony into the tube and resuspend it in the reagent.
  - (3) Perform Steps 3 - 5 of the protocol in Section VI-1.
  
- From liquid culture
  - (1) Centrifuge 1.3 ml of culture at 1,000 rpm for 1 min and transfer 1.0 - 1.2 ml of the bacteria-containing supernatant to a new tube. (This step removes contaminants.)
  - (2) Centrifuge at 12,000 - 15,000 rpm for 3 min and discard the supernatant. Use the bacterial pellet for the next step.
  - (3) Perform Steps 2 - 5 of the protocol in Section VI-1.

**VII. Experimental Examples**

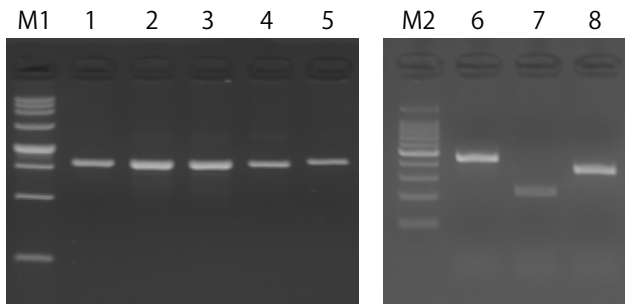
**A. DNA extraction from various microorganisms**

DNA extracts were prepared from colonies of various microorganisms (five bacterial and three fungal species). These extracts were used as templates for PCR with the following kits:

- Bacterial 16S rDNA PCR Kit Fast (800) (Cat. #RR182A)\*
- Fungal rDNA (ITS1) PCR Kit Fast (Cat. #RR183A)\*

\*: Not available in all geographical locations. Check for availability in your region.

DNA extracts prepared from all of the microorganisms tested can be used as PCR templates with these kits.



M1 : 250 bp DNA Ladder  
 1 : *Salmonella enterica* var. Enteritidis  
 2 : *Bacillus subtilis*  
 3 : *Enterococcus faecalis*  
 4 : *Staphylococcus aureus*  
 5 : *Escherichia coli*  
 M2 : 100 bp DNA Ladder  
 6 : *Candida boidinii*  
 7 : *Candida tropicalis*  
 8 : *Saccharomyces cerevisiae*

	<b>Bacterial 16S rDNA PCR Kit Fast (800)</b>	<b>Fungal rDNA (ITS1) PCR Kit Fast</b>
DNA	2.5 $\mu$ l	2.5 $\mu$ l
Total volume	25 $\mu$ l	25 $\mu$ l
Target	1 - 5: 16S rDNA (~800 bp)	6 - 8: ITS1 (150 - 500 bp)
PCR conditions	94°C 5 sec 55°C 1 sec 68°C 4 sec	94°C 5 sec 50°C 1 sec 68°C 3 sec

**B. DNA extraction from mouse tail**

DNA extracts were prepared from 2-mm mouse tail samples using either this product or Company A reagent. Each DNA extract was subjected to PCR using the following enzymes:

- MightyAmp DNA Polymerase Ver.3 (Cat. #R076A/B)\*
- Tks Gflex DNA Polymerase (Cat. #R060A/B)\*

\*: Not available in all geographical locations. Check for availability in your region.



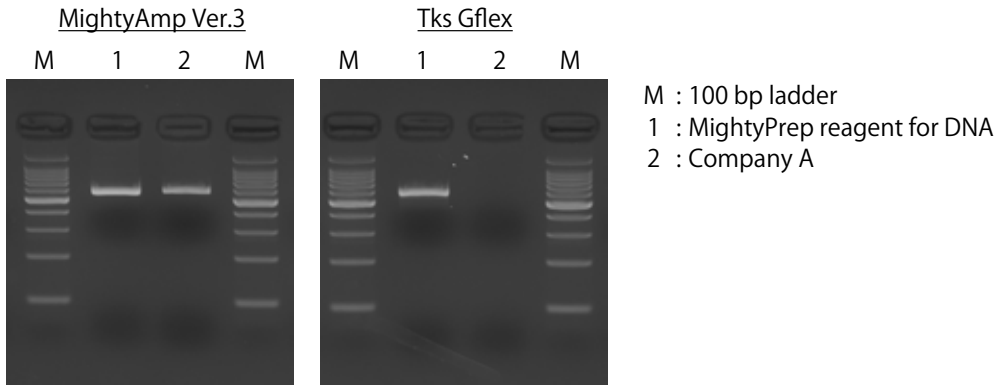
	<b>MightyAmp DNA Polymerase Ver.3</b>	<b>Tks Gflex DNA Polymerase</b>
DNA	2.5 $\mu$ l	2.5 $\mu$ l
Total volume	25 $\mu$ l	25 $\mu$ l
Target	<i>Hbb-b1</i> (542 bp)	<i>Hbb-b1</i> (542 bp)
PCR conditions	98°C 2 min 98°C 10 sec 60°C 15 sec 68°C 30 sec	98°C 10 sec 60°C 15 sec 68°C 15 sec
	} 30 cycles	} 30 cycles

**C. DNA Extraction from *Salmonella* in milk**

DNA extracts were prepared from 10- $\mu$ l samples of milk containing *Salmonella*, using either this product or Company A reagent. The DNA extracts were analyzed for the presence of the *Salmonella invA* gene using the following PCR enzymes:

- MightyAmp DNA Polymerase Ver.3 (Cat. #R076A/B)\*
- Tks Gflex DNA Polymerase (Cat. #R060A/B)\*

\*: Not available in all geographical locations. Check for availability in your region.



	<b>MightyAmp DNA Polymerase Ver.3</b>	<b>Tks Gflex DNA Polymerase</b>
DNA	2.5 $\mu$ l	2.5 $\mu$ l
Total volume	25 $\mu$ l	25 $\mu$ l
Target	<i>invA</i> (605 bp)	<i>invA</i> (605 bp)
PCR conditions	98°C 2 min 98°C 10 sec 60°C 15 sec 68°C 40 sec	98°C 10 sec 60°C 15 sec 68°C 20 sec
	} 30 cycles	} 30 cycles

**VIII. Related Products**

- MightyAmp® DNA Polymerase Ver.3 (Cat. #R076A/B)\*
- Tks Gflex™ DNA Polymerase (Cat. #R060A/B)\*
- NucleoSpin Tissue (Cat. #740952.50/.250)
- Bacterial 16S rDNA PCR Kit Fast (800) (Cat. #RR182A)\*
- Fungal rDNA (ITS1) PCR Kit Fast (Cat. #RR183A)\*

\*: Not available in all geographical locations. Check for availability in your region.

MightyAmp is a registered trademark of Takara Bio, Inc.  
Tks Gflex is a trademark of Takara Bio, Inc.

---

**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 77 565 6973 or from our website at [www.takara-bio.com](http://www.takara-bio.com).

Your use of this product is also subject to compliance with any applicable licensing requirements described on the product web page. It is your responsibility to review, understand and adhere to any restrictions imposed by such statements.

All trademarks are the property of their respective owners. Certain trademarks may not be registered in all jurisdictions.

---