LABISKOMA



# EzWay™ Direct PCR Buffer

## Instruction Manual

Catalog Nos.
K0568001
K0568002

version 201301

## Table of Contents

## I. General Information

EzWay™ Direct PCR Buffer enables the direct amplification of DNA fragments from a variety of samples including whole blood, blood collected on card, saliva, bacteria, mouse tail, tissue, cultured cells or plant eliminating the need for DNA purification, just by adding to the PCR mixture in place of general 10X PCR Buffer.

EzWay™ Direct PCR Buffer is compatible with most commercially available any DNA polymerases including Taq, Pfu, Pwo, Tth and even with any type of Hot Start Taq DNA polymerase. A one-step reaction using this EzWay™ Direct PCR Buffer enables easy PCR and minimizes the sample cross-contamination when dealing with multiple tissue or samples by eliminating tedious genomic DNA preparation.

- No DNA extraction necessary at all - save time, cost, labor
- Direct PCR from Whole Blood, Blood Stains, Blood Cards, Buccal Swab, Saliva, Hair root, sperm, Body Fluid, Cultured Cells and tissues without DNA extraction
- Compatible with various sources of thermostable DNA polymerases (e.g. Taq, Pfu, Pwo, Tth DNA polymerase, all types of hot start DNA polymerase)
- Only small volumes of blood are necessary (0.5-1 µL)
- Reduce the risk of contamination
- Easy PCR Handling and Automation

**Applications**

- Viral infection / Molecular diagnostic test
- Forensic DNA Analysis / Single cell diagnostics
- Blood banking / Identity testing / Multiplex PCR / SNP detection / PCR-RFLP
- Sequencing / Cloning / Laboratory Automatic PCR

## II. Product Contents

| | **EzWay™ Direct PCR Buffer** | **EzWay™ Direct PCR Buffer Set** |
|---|---|---|
| **Cat. No.** | **K0568001** | **K0568002** |
| 5X EzWay™ Direct PCR Buffer, 500ul | O | O |
| 2.5X Direct Lysis Buffer I, 1.5ml | | O |
| 1X Direct Lysis Buffer II, 1.5ml | | O |
| 4X Magic Buffer, 1ml | | O |

**Note:**

a. Most of commercially available Taq Polymerases can be used with K0568001 or K0568002 for Direct PCR application.
b. Simply substitute 10X conventional PCR buffers with 5x EzWay™ Direct PCR Buffer.
(ex. If 5 µL of 10X general PCR buffers is used with 50 µL PCR, then use 10 µL of 5X EzWay™ Direct PCR Buffer.)
c. Direct Lysis Buffer II is for modified alkaline lysis. It is precipitated at low temperature. Use it after completely dissolved. Direct Lysis Buffer II may not be compatible with chemically modified hot start Taq.
d. We recommend that the volume added of Magic Buffer should not exceed 25% (v/v) of the final PCR volume.

**Number of Applications:** 50 reactions at 50 µL PCR Volume / 125 reactions at 20 µL PCR volume

**Storage:** 6 months at 4°C, 1 year at -20°C

## III. Sample Treatment & Template Preparation

### A. Whole Blood

1. Collect blood into tubes containing anticoagulant.
2. Use 0.5-2.5 µL of blood directly into the 25 µL PCR reaction mixture.

   * Alternatively for Lysis method, mix the following reagents and samples, and incubate according to the condition mentioned in the table. After incubation, spin down briefly, then use 1 µL supernatant of lysate for 25 µL PCR.

| Sample | Starting volume | 2.5X Direct Lysis Buffer I | Proteinase K (20 mg/mL) | D.W. | Incubation |
|---|---|---|---|---|---|
| Blood | 2-10 µL | 80 µL | - | 120 µL | 95℃ 15min |
| | 5-20 µL | 80 µL | 2 µL | 120 µL | 56℃ 2hr, then 95℃ 15 min |

### B. Dried Blood/Saliva on the papers (FTA, 903 Card, Guthrie Card, Filter Papers)

1. Collect the blood sample onto a collection card or filter paper and allow drying completely.
2. Punch a disk out of the sample area n the paper using Harris Uni-Core 1.2mm proper punchers.
3. Use 1-2 discs directly into the 25 µL PCR reaction mixture.

### C. Serum/Plasma

1. Place 1-5 µL of 1x Direct Lysis Buffer II into a microcentrifuge tube.
2. Add the same amount of serum/plasma and mix thoroughly by vortexing or pipetting.
3. Incubate at 95℃ for 10 minutes.
4. Use 0.5-2.5 µL of heat treated sample directly into the 25 µL PCR reaction mixture.

### D. Buccal Swab

***** Only Lysis method is available.

1. Collect buccal cells on swab and mix the following reagents and samples, and incubate according to the condition mentioned in the table. After incubation, spin down briefly, then use 1 µL supernatant of lysate for 25 µL PCR.

| Sample | Starting volume | 2.5X Direct Lysis Buffer I | Proteinase K (20 mg/mL) | D.W. | Incubation |
|---|---|---|---|---|---|
| Buccal Swab | 1 cotton | 80 µL | - | 120 µL | 95℃ 15min |
| | 1 cotton | 80 µL | 2 µL | 120 µL | 56℃ 2hr, then 95℃ 15 min |

### E. Saliva/Sputum/Bronchial Lavage (BAL)/Urine

1. Collect 1mL saliva / 1mL sputum (after liquefied) / 15-35 mL bronchial lavage fluid / 10mL urine.
2. Centrifuge at 10,000 x g for 10 minutes and resuspend the cell pellet in 50 µL of TE Buffer or distilled water.
3. Use 1 µL solution directly into the 25 µL PCR reaction mixture.
   * Alternatively for Lysis method, mix the following reagents and samples, and incubate according to the condition mentioned in the table. After incubation, spin down briefly, then use 1 µL supernatant of lysate for 25 µL PCR.

| Sample | Starting volume | 2.5X Direct Lysis Buffer I | Proteinase K (20 mg/mL) | D.W. | Incubation |
|---|---|---|---|---|---|
| Saliva/Sputum /BAL/Urine | Precipitation | 80 µL | - | 120 µL | 95°C 15min |
| | Precipitation | 80 µL | 2 µL | 120 µL | 56°C 2hr, then 95°C 15 min |

**F. Cultured Cells**

1. Collect < 1mL of floating cells by pipetting or <1mg of adhesive cells by scratching or trypsin treatment.
2. Centrifuge at 10,000x g for 10 minutes. Resuspend cell pellet in 50 µL of TE buffer or distilled water.
3. Use 1 µL of the solution directly into the 25 µL PCR reaction mixture.

* Alternatively for Lysis method, mix the following reagents and samples, and incubate according to the condition mentioned in the table. After incubation, spin down briefly, then use 1 µL supernatant of lysate for 25 µL PCR.

| Sample | Starting volume | 2.5X Direct Lysis Buffer I | Proteinase K (20 mg/mL) | D.W. | Incubation |
|---|---|---|---|---|---|
| Cultured Cell | Precipitation | 80 µL | - | 120 µL | 95°C 15min |
| | Precipitation | 80 µL | 2 µL | 120 µL | 56°C 2hr, then 95°C 15 min |

**G. Mouse Tail/Ear**

* We recommend that EzWay™ Mouse Tail Direct PCR Kit (Cat# K0568600) is used for direct PCR from mouse tail/ear.

**H. Animal Tissues**

***** Only Lysis method is available.

1. Mix the following reagents and samples, and incubate according to the condition mentioned in the table. After incubation, spin down briefly, then use 1 µL supernatant of lysate for 25 µL PCR.

| Sample | Starting volume | 2.5X Direct Lysis Buffer I | Proteinase K (20 mg/mL) | D.W. | Incubation |
|---|---|---|---|---|---|
| Tissue | 1-5 mg | 80 µL | - | 120 µL | 80°C 15min |
| | 6-10mg | 80 µL | 2 µL | 120 µL | 56°C 2hr, then 95°C 15 min |
| Ground tissue | 60 uL | 80 µL | - | 60 µL | 80°C 15min |
| | 120 uL | 80 µL | 2 µL | - | 56°C 2hr, then 95°C 15 min |

**I. FFPE Formalin-fixed paraffin-embedded) Tissues**

***** Under development of improved procedures

**J. Hair root**

***** Only Lysis method is available.

1. Mix the following reagents and samples, and incubate according to the condition mentioned in the table. After incubation, spin down briefly, then use 1 µL supernatant of lysate for 25 µL PCR.

| Sample | Starting volume | 2.5X Direct Lysis Buffer I | Proteinase K (20 mg/mL) | D.W. | Incubation |
|---|---|---|---|---|---|
| Hair | 1-2 roots | 80 µL | - | 120 µL | 95°C 30min |
| | 2-4 roots | 80 µL | 2 µL | 120 µL | 56°C 2hr, then 95°C 30 min |

**K. Plant**

* We recommend that EzWay™ Plant Direct PCR Kit (Cat# K0568700) is used for direct PCR from plant.

**L. Bacteria / Virus from various sources**

***** Under development of improved procedures

**M. Feces with occult blood**

1. Collect 50mg feces/stool and suspend in 10 times volume (w/v) of PBS and crush.
2. Centrifuge at 3000 g for 1 minute.
3. Collect the supernatant and Incubate at 95°C for 5 minutes.
4. Use 1ul of solution directly into the 25 µL PCR reaction mixture.

* Alternatively for Lysis method, mix the following reagents and samples, and incubate according to the condition mentioned in the table. After incubation, spin down briefly, then use 1 µL supernatant of lysate for 25 µL PCR.

| Sample | Starting volume | 2.5X Direct Lysis Buffer I | Proteinase K (20 mg/mL) | D.W. | Incubation |
|---|---|---|---|---|---|
| Feces | 100 µL | 80 µL | - | 120 µL | 95°C 15min |
| | 100 µL | 80 µL | 2 µL | 120 µL | 56°C 2hr, then 95°C 15 min |

## IV. PCR Amplification

1. Add the following reagents to a thin-walled PCR microcentrifuge tube or plate.

| Component | Final Concentration | Volume/reaction | |
|---|---|---|---|
| 5X EzWay™ Direct PCR Buffer | 1x | 5 µL | 10 µL |
| dNTP mix (2.5 mM each) | 200uM of each dNTP | 2 µL | 4 µL |
| 5' Primer | 0.1 - 0.5 uM | Variable | Variable |
| 3' Primer | 0.1 - 0.5 uM | Variable | Variable |
| Taq DNA Polymerase (5U/µL) | - | 0.25 µL | 0.5 µL |
| Distilled water | - | Variable | Variable |
| Template | - | Variable (0.2-1 µL) | Variable (0.5-2 µL) |
| **Total reaction volume** | | **25 µL** | **50 µL** |

2. Mix gently.

3. When using a thermal cycler without a heated lid, add approximately 100 µL of mineral oil on top of the mixture.

4. Perform thermal cycling.

| Step | | Temp. | Time | Cycles |
|---|---|---|---|---|
| **Initial Denaturation** | | 95℃ | 5-15 min | 1 |
| **Cycling** | Denaturation | 94℃ | 0.5-1 min | 35-45 |
| | Annealing | 50-68℃ | 0.5-1 min | |
| | Extension | 72℃ | 1-5 min (~1kb/imin) | |
| **Final Extension** | | 72℃ | 10min | 1 |

**Note:**

a. Primers should be 15 to 30 bases in length and near 50% G+C content.
b. Magic Buffer will improve DNA amplification of templates that have a high G+C content and a high degree of secondary structure. We recommend that the volume added should not exceed 25 %(v/v) of final PCR volume.
c. If chemically modified hot start Taq PCR enzyme is used, the best initial denaturation time is 10-15 minutes at 95°C.

5. The amplified DNA can be detected by various electrophoresis techniques.
   The most common techniques are agarose or polyacrylamide gel electrophoresis depending on the size of the amplicon.
   **TAE gel is highly recommended** because the bands may be detected dispersed and/or distorted in TBE gel.

## V. Troubleshooting

| Problem | Probable Cause | Solution |
|---|---|---|
| No or weak PCR Products | PCR may be inhibited by components in the sample | Dilute sample 10 fold serially (1, 10, 100,… fold) with 1x TE Buffer for Direct Method. Reduce starting sample amount 10 fold serially (1, 10, 100,… fold) for Lysis Method. Do not change lysed sample volume for PCR. |
| | $MgCl_2$ is not optimized | Optimize $MgCl_2$ concentration between 1.5 ~ 4.0 mM in 0.5 mM increments. |
| | One or more components may be missing | First, confirm that proper volume of EzWay™ PCR Buffer was used instead of general PCR buffer.<br>Always run a positive control using known purified DNA. |
| | Inadequate lysis | Adjust sample amount, lysis volume and time for full lysis.<br>Check that the correct lysis mixture is compromised.<br>More proteinase-K should be added. DTT should be added to lysis mixture necessarily for hair root, sperm lysis. |
| | PCR conditions are not optimized. (The annealing temperature may be too high; More cycles may be needed; The denaturation or extension time may be too short. | The gradient PCR may help the first PCR parameter setting. If it is not impossible, optimize the PCR conditions by changing annealing temperature, or the cycle numbers, or extension time. It is recommended to change one parameter each time. |
| | The primers are not optimally designed. | Longer primers of 25-30nts with a GC content of 45-60% and with a more stable 3'-end usually gives better result. |
| | High GC contents. | Recommend to use Magic buffer. |
| | Genomic material may be lost especially when a single cell is used. | Do not lysis a single. Directly add PCR mixture to cell (contained solution). |
| Non-specific DNA products | The primers are not optimally designed. | Longer primers of 25-30nts with a GC content of 45-60% and with a more stable 3'-end usually gives better result. |
| | Annealing temperature is too low. | Optimize the PCR conditions by increasing annealing temperature in 2-4℃ increments, or decreasing the number of cycles. |
| High background | Too much DNA polymerase may be used | Decrease the amount of the DNA polymerase gradually. |
| False positive | Reagents are contaminated | It is recommended that a negative control without template. Recombinant DNA polymerase may contain a low amount of *E.coli* DNA and may be the major cause of contamination especially when *E.coli* PCR. |