# EzWay™
# Direct Taq PCR MasterMix

Catalog Nos.
K0568010
K0568020

# Direct Hot Taq PCR MasterMix

Catalog Nos.
K0568210

# Direct Hot Universal qMasterMix

Catalog Nos.
K0569020

# Direct Multiplex PCR MasterMix

Catalog Nos.
K0567840

## Instruction Manual

## Table of Contents

## I. General Information

Direct PCR Technology provides robust PCR performance in a wide range of PCR applications without the need for time-consuming optimization. It allows direct PCR from all types of anticoagulant-treated bloods (heparin, EDTA, sodium citrate), plasma, serum, buffy coat, buccal swab without any DNA purification steps. Also, it can be used for direct PCR of various samples such as tissues, paraffin-embedded tissue slide or array, cultured cells, body fluids, bacteria, viruses, plants, yeast, hairs and so on.

- 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, Bacteria, Viruses, and Tissues (fresh, frozen or paraffinized) without DNA extraction
- Only small volumes of sample are used.
- Reduce the risk of contamination
- Easy PCR Handling and Automation

EzWay™ Direct Taq PCR MasterMix is a premixed solution containing Taq DNA Polymerase, dNTPs, $MgCl_2$, Direct PCR Buffer, additives in a 1.5ml tube. This product has two versions: with dye or without dye.

EzWay™ Direct Hot Taq PCR MasterMix is a premixed solution containing Hot Taq DNA Polymerase, dNTPs, $MgCl_2$, Direct PCR Buffer, dye and additives in a 1.5ml tube. Hot Taq DNA Polymerase, a chemically modified form of Taq DNA Polymerase, provides high specificity in hot-start PCR.

EzWay™ Direct Hot Universal qMasterMix is a premixed solution containing Hot Taq DNA Polymerase, dNTPs, $MgCl_2$, and Direct PCR Buffer optimized for real time PCR in a 1.5ml tube.

### 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

| Cat. No. | Purpose | K0568010 | K0568020 | K0568210 | K0569020 | K0567840 |
|---|---|---|---|---|---|---|
| 2X EzWay™ Direct Taq PCR MasterMix w/ dye, 1ml | Routine PCR | O | | | | |
| 2X EzWay™ Direct Taq PCR MasterMix w/o dye, 1ml | Routine PCR | | O | | | |
| 2X EzWay™ Direct Hot Taq PCR MasterMix W/ dye, 1ml | Hot Start PCR | | | O | | |
| 2X EzWay™ Direct Hot Universal qMasterMix, 1ml | Real time qPCR | | | | O | |
| 2X EzWay™ Direct Multiplex PCR MasterMix, 1ml | Multiplex PCR | | | | | O |
| 4X Magic Buffer, 1ml | High GC amplification | O | O | O | O | O |

**Note:**

a. Direct Lysis Buffer I/II and Direct Elution Buffer are not included in this product, so they must be purchased or prepared separately for the Lysis Method depending on samples.
b. All MasterMix packages contain 4X Magic Buffer (K0561031) for amplification of high GC contents.

**Number of Applications:**
40 reactions at 50ul PCR Volume / 100 reactions at 20ul PCR volume

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

## III. Sample Treatment & Template Preparation

### A. Whole Blood

For end point PCR

1. Collect blood into tubes containing anticoagulant.
2. Use 1 µL of blood directly into the 50 µL PCR reaction mixture.

For real time PCR

1. Collect blood into tubes containing anticoagulant .
2. Remove the RBC's with RBC lysis method and resuspend the WBC's precipitate homogeneously with the same volume of D.W.
3. Use 1 µL of blood directly into the 50 µL PCR reaction mixture.

### B. Dried Blood

For end point PCR

1. Collect the blood sample onto a collection card or filter paper and allow to dry completely.
2. Punch a disk (preferably 1.2 mm diameter) or cut the filter paper into 1x1mm squares and place into a microcentrifuge tube.
3. Add 50-100 µL of 1x Elution Buffer for 20 min.
   Use 1-5 µL of eluant solution directly into the 50 µL PCR reaction mixture.

For real time PCR

1. Collect the blood sample onto a collection card or filter paper and allow to dry completely.
2. Punch a disk (preferably 1.2 mm diameter) or cut the filter paper into 1x1mm squares and place into a microcentrifuge tube.
3. Add 50-100 µL of 1x Elution Buffer for 20 min.
4. Remove the RBC's with RBC lysis method and resuspend the WBC's precipitate homogeneously with the same volume of D.W.
5. Use 1-5 µL of eluant solution directly into the 50 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 0.5-5 µL of heat treated sample directly into the 50 µL PCR reaction mixture.

### D. Buccal Swab

1. Collect buccal cells on swab and allow the swab to dry.
2. Immerse the dried buccal swab into 10 mL PBS for 20 min.
3. Use 1 µL of eluant solution directly into the 50 µL PCR reaction mixture.

### E. Saliva

1. Use 0.25-5 µL of saliva directly into the 50 µL PCR reaction mixture.

**F. Cultured Cells**

1. Grow monolayer cells in a plate until 90~95% confluent.
2. Aspirate the medium from the wells using a pipette tip connected to the vacuum system. The medium must be removed completely.
3. Add 20 μL of the 1x Direct Lysis Buffer I or II to the wells.
4. Incubate the plate at 75℃ for 5-10 minutes.
5. Use 1 μL of the supernatant directly into the 50 μL PCR reaction mixture.

**G. Mouse Tail ***

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

**H. Animal Tissues ***

1. Place a 1-10 mg piece of tissue into the 1x Direct Lysis Buffer I and Proteinase K. (Refer to Appendix II for the lysis condition.)
2. Incubate at 55℃ for 2 hours.
3. Incubate at 95°C for 5 minutes and Spin down briefly.
4. Use 1 μL of supernatant of lysate directly into the 50 μL PCR reaction mixture.

**I. FFPE Tissues**

For end point PCR

1. Deparaffinize tissue sections and scratch up the tissue of interest with syringe needle.
2. Immerse the edge of the needle covered with the tissue powders directly into the 50 μL PCR reaction mixture.

For real time PCR

1. Deparaffinize tissue sections and destain it with the proper destaining method.
2. Scratch up the tissue of interest with syringe needle.
3. Immerse the edge of the needle covered with the tissue powders directly into the 50 μL PCR reaction mixture.

**J. Hair root / Sperm***

1. Collect 1-2 roots of samples into the 1x Direct Lysis Buffer I, dithiothreitol (DTT) and Proteinase K. (Refer to Appendix II for the lysis condition.)
2. Incubate at 55℃ for 3 hours.
3. Incubate at 95°C for 5 minutes and Spin down briefly.
4. Use 1ul of supernatant of lysate directly into the 50 μL PCR reaction mixture.

**Note:** If hair roots lysate contain color pigments, real time PCR can not be applied.

**K. Sputum / Bronchial Lavage Fluid / Urine**

1. Collect 1ml sputum or 15-35 mL bronchial lavage fluid or 10ml urine and centrifuge at 10,000 x g for 10 minutes.
2. Resuspend the cell pellet in 50 μL of 1x Elution Buffer.
3. Use 1 μL solution directly into the 50 μL PCR reaction mixture.

**L. Plant***

1. Collect 1-10 mg of plant tissue and disrupt using a mortar and pestle.
2. Add 100-200 µL of the 1x Direct Lysis Buffer I.
3. Incubate at 80°C for 2 hours and allow to cool on ice.
4. Centrifuge at 6000 rpm for 5 minutes.
5. Use 1 µL of supernatant of lysate directly into the 50 µL PCR reaction mixture.

**M. Bacteria/Virus from Feces (Gram-negative bacteria)**

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

**N. Bacteria/Virus from Feces* (Gram-positive bacteria)**

1. Collect 10mg 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 add 1x Direct Lysis Buffer I and Proteinase K. (Refer to Appendix II for the lysis condition.)
4. Incubate the tubes at 55°C for 2 hours.
5. Incubate at 85°C for 45 minutes and Spin down briefly.
6. Use 1ul of supernatant directly into the 50 µL PCR reaction mixture.

**Note:**

a. For samples marked with asterisk (*), the lysis step is required. Lysis Buffer can be purchased separately.
b. If the sample contains color pigments, real time PCR can not be applied, because it may interfere with the reaction.
c. All the Lysis method requires to use Proteinase K.
d. Refer to the suggested starting lysis conditions per sample at 9. Appendix II.

## IV. PCR Amplification

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

| Component | Volume/reaction | |
|---|---|---|
| 2x EzWay™ Direct Master Mix or Direct Hot MasterMix | 10 μL | 25 μL |
| 5' Primer (final conc. 0.1 - 0.5 uM) | Variable | Variable |
| 3' Primer (final conc. 0.1 - 0.5 uM) | Variable | Variable |
| Distilled water | Variable | Variable |
| Template | Variable (0.2-2 μL) | Variable (0.5-5 μL) |
| **Total reaction volume** | **20 μL** | **50 μL** |

For real time PCR

| Component | Volume/reaction | |
|---|---|---|
| 2X EzWay™ Direct Hot Universal qMasterMix | 10 μL | 25 μL |
| 5' Primer (final conc.0.2 - 0.4 uM) | Variable | Variable |
| 3' Primer (final conc.0.2 - 0.4 uM) | Variable | Variable |
| Probe (final conc.0.1 - 0.2 uM) | Variable | Variable |
| Distilled water | Variable | Variable |
| Template | Variable (0.2-2 μL) | Variable (0.5-5 μL) |
| **Total reaction volume** | **20 μL** | **50 μL** |

**Note:**
a. Make sure the sample doesn't contain any pigment.
b. Primers should be 15 to 30 bases in length and near 50% G+C content.
c. 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.

2. Mix gently.

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

4. Perform thermal cycling.

For routine PCR

| Step | | Temp. | Time | Cycles |
|---|---|---|---|---|
| **Initial Denaturation** | | 95℃ | 5 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 |

For Hot Start PCR

| Step | | Temp. | Time | Cycles |
|---|---|---|---|---|
| **Initial Denaturation** | | 95℃ | 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 |

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.

For real time PCR

| Step | | Temp. | Time | Cycles |
|---|---|---|---|---|
| **Initial Denaturation** | | 95℃ | 5 min | 1 |
| **Cycling** | Denaturation | 95℃ | 15sec | 45-50 |
| | Annealing & Extension | 55-60℃ | 1min (fluorescence detection) | |

For melting curve analysis, specified temperature range (e.g. 50-95℃) can be added to the final step while fluorescence is detected.

## V. Appendix

Appendix I. Cautions when preparing a sample

| | |
|---|---|
| Blood | Too much concentrated anticoagulant in the blood might cause PCR inhibition. When blood samples are collected into vacutainer tubes, check enough amount of blood (at least > 2ml) was collected as described on the surface of the tube.<br><br>When lysis method is used, heating step at 85°C for 45 minutes must be performed to inactivate the Proteinase K.<br><br>Since Direct Lysis Buffer I contains highly concentrated EDTA and SDS, high amount of lysate may not get a result.<br><br>Direct Lysis Buffer II is an alkaline solution, so it may not be compatible with chemically modified hot start Taq. |

Appendix II. Lysis conditions for Lysis method

| Sample | Starting volume/ amount for lysis | 5X Direct Lysis Buffer I | Proteinase K (20 mg/mL) | DTT (1 M) | D.W. |
|---|---|---|---|---|---|
| Blood | 1-5 µL | 10 µL | 1 µL | - | 39 µL |
| | 6-10 µL | 20 µL | 2 µL | - | 78 µL |
| Saliva | 100 µL | 25 µL | 1 µL | - | - |
| Mouse Tail | 1-2 mm | 20 µL | 1 µL | - | 75 µL |
| | 3-4 mm | 40 µL | 2 µL | - | 150 µL |
| Tissue Slide | 5 µL elution/ region | 10 µL | 5 µL | - | 30 µL |
| | 10 µL elution/ region | 20 µL | 10 µL | - | 60 µL |
| Tissue Microarray | 1 µL elution/ 1-1.5mm spot | 2 µL | 1 µL | - | 6 µL |
| | 4 µL elution/ 2-4mm spot | 4 µL | 2 µL | - | 10 µL |
| Tissue/Organ | 1-5 mg | 20 µL | 1 µL | - | 79 µL |
| | 6-10 mg | 40 µL | 2 µL | - | 158 µL |
| | 100 µL | 25 µL | 0.25 µL | - | - |
| Hair root/Sperm | 1-2 root | 10 µL | 1 µL | 2 µL | 37 µL |
| | 3-8 root | 20 µL | 2 µL | 4 µL | 74 µL |
| Bacteria from feces | 10 mg of Feces + 10 vol. of PBS | 40 µL | 20 µL | - | 20 µL |
| Gram(+) Bacteria | 1 colony | 20 µL | 10 µL | - | 60 µL |
| | $10^3$-$10^4$ CFU | 20 µL | 10 µL | - | 60 µL |
| | $10^5$-$10^8$ CFU | 40 µL | 20 µL | - | 120 µL |
| Sputum/BAL/Urine | - | 25 µL | 1 µL | - | 74 µL |
| Plant | 1-10 mg | 20 µL | - | - | 80 µL |

## VI. 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 Elution 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. Check that the correct lysis mixture is compromised. Incomplete deparaffinized tissue will give worse result. 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. | Betaine, DMSO, formamide or TMAC can help the problem of high GC-rich template. |
| | 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. |

## VII. References

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3. Yang,Y.G., Kim,J.Y., Soh,M.S. and Kim,D.S. (2007) A simple and rapid gene amplification from Arabidopsis leaves using AnyDirect system. *J. Biochem. Mol. Biol.*, **40**, 444-447.

4. Yang,Y.G., Song,M.K., Park,S.J. and Kim,S.W. (2007) Direct detection of Shigella flexneri and Salmonella typhimurium in human feces by real-time PCR. *J. Micobiol. Biotechnol.*, **17**, 1616-1621.