Contents

Introduction. 2

Overview. 2

Storage and Stability. 2

Kit Contents. 3

Materials to Be Provided by User. 3

Before Starting. 3

DNA Extraction from Whole Blood. 4

DNA Extraction from Serum or Plasma. 4

DNA Extraction from Dried Blood Spot. 5

DNA Extraction from Saliva. 6

DNA Extraction from Dried Saliva Spot 6

DNA Extraction from Buccal Swab. 7

Troubleshooting Guide. 8

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Introduction

The E.Z.N.A.™ Direct PCR kits are an innovative buffer systems that radically simplifies the extraction of nucleic acids from a variety of sources. These kit contents reagents that quickly release the genomic DNA from samples and neutralize the inhibitors. The lysate is ready for PCR amplification without any further purification procedure.

Overview

The E.Z.N.A.™ Blood Direct PCR Kit provides an easy and rapid method for the extraction of genomic DNA for consistent PCR analysis. Sample (Include fresh, frozen whole blood, dried blood spot, saliva, dried saliva spot and buccal swab) is first lysed in lysis Buffer (B1 Buffer), after a short incubation, the lysate is neutralized with B2 buffer. The lysate is then ready for PCR amplification.

Storage and Stability

All components of the E.Z.N.A.™ Direct Blood PCR kit can be stored at room temperature. The kit is guaranteed for 12 moths.

Kit Contents

Product	TQ2300-00	TQ2300-01	TQ2300-02
Purification	20 Preps	100 Preps	500 Preps
Buffer B1	1.2 mL	6 mL	30 mL
Buffer B2	1.2 mL	6 mL	30 mL
Direct PCR Buffer (10x)	10 mL	50 mL	250 mL
User Manual	1	1	1

Materials to Be Provided by User

- Tabletop microcentrifuge
- Nuclease-free 1.5 mL tubes
- Water bath or heating block set to 55°C (for dried blood or saliva spot)

Before Starting

- Please read the entire booklet to become familiar with the E.Z.N.A.™ Direct Blood PCR procedure.
- Choose the most appropriate protocol to follow. Protocols are described for each of samples.
- Follow general lab protection procedures such as wearing gloves, safty glass when handling any reagent supplied with the kit. Avid contact skin.

DNA Extraction From Whole Blood

All steps should be carried out at room temperature unless otherwise noted.

1. Transfer 50 µl Buffer B1 to a 1.5 ml microcentrifuge tube.
2. Add 5-10 µl whole blood into the tube contains 50 µl Buffer B1.
3. Mix throughly by vorexing at maximum speed for 20 seconds.
4. Incubate at room temperature for 10-15 minutes.
5. Add 50 µl Buffer B2 and mix throughly by vorexing or pipetting.
6. Store the lysate at 4°C
7. PCR amplification: Add 1-5 µl lysate to 20-50 µl reaction using Direct PCR Buffer (supplied). Add primers, Taq polymerase, dNTP and $MgCl_2$ for PCR amplification. **Note:** the volume of lysate should not exceed 10% of total PCR reaction volume.

DNA Extraction From Serum or plasma

All steps should be carried out at room temperature unless otherwise noted.

1. Transfer 50 µl Buffer B1 to a 1.5 ml microcentrifuge tube.
2. Add 5-10 µl serum or plasma into the tube contains 50 µl Buffer B1.
3. Mix throughly by vorexing at maximum speed for 20 seconds.
4. Incubate at room temperature for 10-15 minutes.
5. Add 50 µl Buffer B2 and mix throughly by vorexing or pipetting.
6. Store the lysate at 4°C

7. Add 1-5 µl lysate to 20-50 µl reaction using Direct PCR Buffer (supplied). Add primers, Taq polymerase, dNTP and $MgCl_2$ for PCR amplification. **Note:** the volume of lysate should not exceed 10% of total PCR reaction volume.

DNA Extraction From Dried Blood Spot

All steps should be carried out at room temperature unless otherwise noted.

1. Transfer 50 µl Buffer B1 to a 1.5 ml microcentrifuge tube.
2. Punch a 15-30 mm^2 dried blood disk (3-4 mm).
3. Add the dried blood disk into the tube contains 50 µl Buffer B1.
4. Mix throughly by vorexing at maximum speed for 30 seconds. Note: Centrifuge for few seconds to bring down liquid drop from tube lid if necessary.
5. Incubate at 55 °C for 10-15 minutes.
6. Add 50 µl Buffer B2 and mix throughly by vorexing or pipetting.
7. Store the lysate at 4°C.
8. Add 1-5 µl lysate to 20-50 µl reaction using Direct PCR Buffer (supplied). Add primers, Taq polymerase, dNTP and $MgCl_2$ for PCR amplification. **Note:** the volume of lysate should not exceed 10% of total PCR reaction volume.

DNA Extraction From Saliva

All steps should be carried out at room temperature unless otherwise noted.

1. Transfer 50 µl Buffer B1 to a 1.5 ml microcentrifuge tube.
2. Add 5-10 µl saliva into the tube contains 50 µl Buffer B1.

3. Mix throughly by vorexing at maximum speed for 30 seconds.
4. Incubate at room temperature for 10-15 minutes.
5. Add 50 µl Buffer B2 and mix throughly by vorexing or pipetting.
6. Store the lysate at 4°C.
7. Add 1-5 µl lysate to 20-50 µl reaction using Direct PCR Buffer (supplied). Add primers, Taq polymerase, dNTP and $MgCl_2$ for PCR amplification. **Note:** the volume of lysate should not exceed 10% of total PCR reaction volume.

DNA Extraction From Dried Saliva Spot

All steps should be carried out at room temperature unless otherwise noted.

1. Transfer 50 µl Buffer B1 to a 1.5 ml microcentrifuge tube.
2. Punch a 15-30 mm^2 dried saliva disk (3-4 mm).
3. Add the dried blood disk into the tube contains 50 µl Buffer B1.
4. Mix throughly by vorexing at maximum speed for 30 seconds. Note: Centrifuge for few seconds to bring down liquid drop from tube lid if necessary.
5. Incubate at 55 °C for 10-15 minutes.
6. Add 50 µl Buffer B2 and mix throughly by vorexing or pipetting.
7. Store the lysate at at 4°C.
8. Add 1-5 µl lysate to 20-50 µl reaction using Direct PCR Buffer

(supplied). Add primers, Taq polymerase, dNTP and $MgCl_2$ for PCR amplification. **Note:** the volume of lysate should not exceed 10% of total PCR reaction volume.

Optional: DNA Extraction From Buccal Swab

Note: The Buccal Swab protocol requires more reagents than stand blood protocols, less preparation can be processed by using this protocol. All steps should be carried out at room temperature unless otherwise noted.

1. Transfer 300 µl Buffer B1 to a 1.5 ml microcentrifuge tube.

2. Cut the buccal swab and place the swab into the tube contains 300 µl Buffer B1.

3. Mix throughly by vorexing at maximum speed for 30 seconds. Note: Centrifuge for few seconds to bring down liquid drop from tube lid if necessary.

4. Incubate at 55 °C for 10-15 minutes.

5. Add 50 µl Buffer B2 and mix throughly by vorexing or pipetting.

6. Store the lysate at at 4°C.

7. Add 1-5 µl lysate to 20-50 µl reaction using Direct PCR Buffer (supplied). Add primers, Taq polymerase, dNTP and $MgCl_2$ for PCR amplification. **Note:** the volume of lysate should not exceed 10% of total PCR reaction volume.

Troubleshooting Guide

Problem	Possible Cause	Suggestions
Little or no PCR product is detected	PCR Reaction is inhibited due to the presence of contaminants in the blood lysate	Use less lysate or diluted the lysate with water and repeat PCR.