RoomTemp Sample Lysis Kit

Catalog# P073

Version 9.1

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01/ Introduction

The RoomTemp Sample Lysis Kit is a simple and fast blood lysis kit used at room temperature. The kit contains two components, Lysis Buffer and Stabilizing Buffer. Lysis Buffer contains special components that rapidly destroy the proteins and structures of cell membrane, and can fully release genomic DNA in cells. Stabilizing Buffer contains protective proteins and stabilizing factors to eliminate the effect of the inhibitors in the lysed sample on the downstream reaction such as qPCR and PCR, allowing long-term storage of the lysed DNA solution. Applicable blood sample types include fresh blood, cryopreserved blood, and conventional anticoagulant blood (EDTA, citrate, sodium heparin, etc.). Genomic DNA can be released from whole blood samples by lysis for 3 min at room temperature. And the lysed DNA solution can be directly used as a template for downstream applications such as SNP detection by probe-based Taqman, quantification by probe-based qPCR, PCR amplification. No need for complicated extraction operations, it can achieve the same effect as the traditional genome extraction method. In addition to blood, this kit is also compatible with a variety of samples such as lysed FTA cards, buccal swabs, and plant tissues.

02/ Components

Components	P073-01 (250 rxn)	P073-02 (1000 rxn)	P073-03 (5000 rxn)
Lysis Buffer	5 ml	20 ml	100 ml
Stabilizing Buffer	5 ml	20 ml	100 ml

03/ Storage

Store at 2°C ~ 8°C. Transport at 2°C ~ 8°C.

04/ Quality Control

Functional assay-quantitative qPCR: use 3 μl of fresh whole blood lysate as a template for amplification in three detection systems (total 20 μl) The amplification curves between batches are similar, and the △Ct value is within ± 0.5.

05/ Workflows

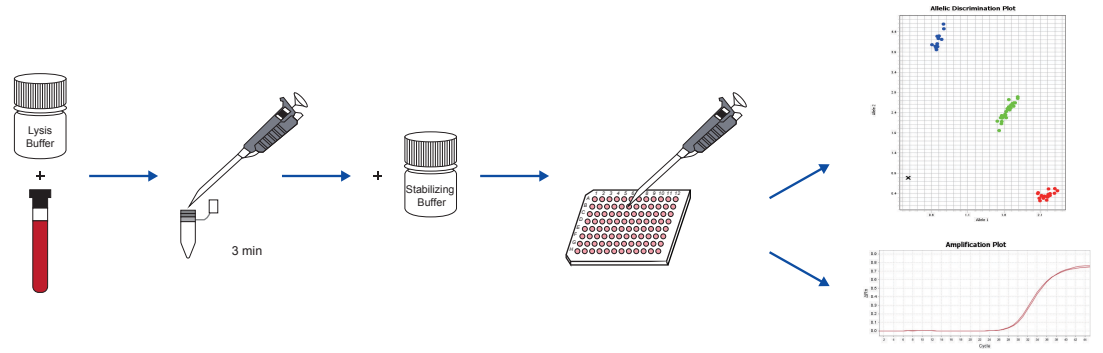

Fig. 1. Workflow of RoomTemp Sample Lysis Kit

06/ Protocol

1. Mix the Lysis Buffer and Stabilizing Buffer slightly before use to avoid large bubbles.

2. Place the sample to be lysed in a centrifuge tube, add an appropriate amount of Lysis Buffer, vortex to mix throughly, and collect the mixture to the bottom of the tube at low speed. (The recommended volume of the sample to be lysed and the Lysis Buffer, please refer to **Table 1**) [a]

3. Incubate for 3 min at room temperature (20°C ~ 25°C) or 95°C. (Please refer to **Table 1** for incubation conditions for different types of samples)

4. Add the equal volume of Stabilizing Buffer to Lysis Buffer, vortex to mix throughly, and collect the mixture to the bottom of the tube at low speed to complete the preparation of the DNA lysis solution. (Please refer to **Table 1** for the recommended volume of Stabilizing Buffer) [b]

5. For 20 μl amplification system, it is recommended to take 1 μl - 4 μl of the lysed DNA solution as a template for downstream detection experiments.

(1) It is recommended to use with ChamQ Geno-SNP Probe Master Mix (Vazyme, #Q811) for probe-based Taqman SNP detection or other tests.

(2) It is recommended to use with AceQ Universal U+ Probe Master Mix V2 (Vazyme, #Q513) for quantification by probe-based qPCR.

(3) It is recommended to use with 2 × Rapid Taq Master Mix (Vazyme, #P222) or 2 × Phanta Max Master Mix (Vazyme, #P515) for PCR amplification.

a. If the sample size is large, please scale up the volume of Lysis Buffer and Stabilizing Buffer. For example, 5 μl of blood can be lysed by adding 50 μl of Lysis Buffer. After incubating for 3 min at room temperature, add 50 μl of Stabilizing Buffer and mix by inversion.

b. The lysed DNA solution can be stored at 4°C for 1 month. For long-term storage, the lysed DNA solution should be frozen at -20°C and mixed by inversion before use.

Table 1. Cleavage Conditions for Different Types of Samples

	Sample Size	The Volume of Lysis Buffer	Incubation Conditions [a]	The Volume of Stabilizing Buffer
Fresh whole blood, whole blood collected by EDTA/citrate/heparin sodium anticoagulant tube[b]	2 μl	20 μl	Room temperature, 3 min	20 μl
Blood stains on Whatman® 903 and FTA® cards	3 mm	50 μl	95°C, 3 min	50 μl
Oral swab[c]	1	400 μl	95°C, 3 min	400 μl
Cell suspension	2 μl	20 μl	Room temperature, 3 min	20 μl
Tissue homogenate	5 μl	50 μl	95°C, 3 min	50 μl
Rat tail	1 - 2 mm	50 μl	95°C, 3 min	50 μl
Hair (with follicles)	2 - 3	50 μl	95°C, 3 min	50 μl
Plant leaves	2 - 3 mm	50 μl	95°C, 3 min	50 μl

a. Room temperature: 20°C ~ 25°C. For the samples that are more difficult to lyse, the treated time can be extended appropriately.

b. The kit has excellent resistance to impurities and is compatible with hyperlipidemia, high bilirubin whole blood and hemolyzed samples.

c. Oral swabs: There are two options for lysing buccal swabs:

Option 1 is to place the collected buccal swabs (contain oral cells) directly into 400 μl of Lysis Buffer, rotate 5 times, squeeze the adsorbed internal solution and then discard the swab. After incubating for 3 min at 95 ° C, add 400 μl of Stabilizing Buffer and mix by inversion.

Option 2, the collected buccal swabs are eluted with a solution such as physiological saline to form a cell suspension. Take 2 μl of the cell suspension to 20 μl of Lysis Buffer, and after incubation at 95 ° C for 3 min, then add 20 μl of Stabilizing Buffer, and mix by inversion.

07/ FAQ & Trouble Shooting

◇ Low plateau in qPCR amplification or low PCR yield.

This may be due to the small amount of sample lysed or the low expression level of the test gene. Try to increase the amount of lysed sample and the template input in the amplification system. Taking blood as an example, the recommended amount of Lysis buffer added in **Table 1** can lyse 2 μl - 10 μl of samples, and the 20 μl of amplification system is compatible with 1 μl - 10 μl of template, and has no effect on subsequent qPCR and PCR reactions.

◇ After the blood sample is lysed, the color appears blood red.

Generally, the solution after blood lysis is brown, but some blood samples appear blood red, which is related to blood samples, which is a normal phenomenon and has no effect on subsequent experiments.

◇ Is it possible to use only Lysis Buffer for lysis and then directly perform subsequent amplification reactions?

Stabilizing Buffer contains protective proteins and stabilizing factors to eliminate the effect of the inhibitors in the lysed sample on the downstream reaction such as qPCR and PCR, allowing long-term storage of the lysed DNA solution. If only Lysis Buffer is used for sample lysis, the recommended template input for downstream amplification in a 20 μl system is 1 μl - 2 μl, and the lysed DNA solution can be stored at -20°C for only 1 week. In other cases, it is not recommended to use only Lysis Buffer.

ISO 9001: 2015