User Guide

Qexp-MDx ALDH2 Genotyping Kit

Research Use Only

For more detailed information, please find it in the Operation Manual. (page number)

A. Sample Collection *(page 9)*

Blood

Please follow the guidelines announced by the local governments for appropriate operation.
Only EDTA Blood Collection Tubes (lavender/purple cap) are available for this kit.

Oral swab

1. Rinse the mouth with clean water for 10 seconds to remove microorganisms, and food or drink remnants.
2. Rub and rotate the brush on the buccal mucosa and gum 10 times on each side to collect cells.
3. Insert the brush into the supplied Collection Tube with UTM.
4. Snap the brush in the tube, then screw the cap of the Collection Tube.
5. Mix well gently to release the cells on the brush into UTM.
   * sample mixture with UTM can be stored at -20℃ for a week.

B. Direct PCR Preparation *(page 10)*

1. Prepare direct PCR master mix reagents as shown in the table below:

Component	Volume per sample	Volume for n samples plus 2 controls	Volume for 46 samples plus 2 controls
Primer mix-ALDH2	2	2 x (n+2)	96
Direct PCR PreMix	12.5	12.5 x (n+2)	600
Nuclease-free water	5.5	5.5 x (n+2)	264
TOTAL (µL)	20	20 x (n +2)	960

2. Transfer 20 µL Master Mix into each of the PCR tubes after mixing it gently.

C. Sample Lysis *(page 10)*

Blood lysate

1. Mix the EDTA Blood Collection Tube well by inverting gently before use.
2. Transfer 5 µL blood sample into a clean PCR tube or microcentrifuge tube.
3. Add 95 µL DNA Lysis Buffer and mix well by pipetting or gently vortex.
4. Incubate at room temperature for 10 mins.

Oral swab lysate

1. Transfer 20 µL sample mixture from the Collection Tube into a clean PCR tube.
2. Add 2.6 µL Proteinase K into the PCR tube and then incubate at room temperature for 1 min.
3. Incubate at 95℃ for 5 mins to inactivate Proteinase K, then cool down to 4℃.
4. Add 20 µL DNA Lysis Buffer (L1) into the PCR tube and mix well by gently vortex or

pipetting.

5. Incubate at room temperature for 10 mins.
6. Add samples as the following table *(page 11)*:

Component	Volume per reaction			
	NTC	Sample reaction	Positive control	Standard sample*
Direct PCR Master Mix	20	20	20	20
specimen lysate	-	5	-	-
Nuclease-free water	5	-	-	3
Positive control	-	-	5	-
Standard Sample	-	-	-	2
TOTAL (μL)	25	25	25	25

D. **PCR Program Set-up** *(page 12)*

Step	Number of cycles	Temperature (℃)	Time
Activation	1	95	10 minutes
Denaturation	35	95	10 seconds
Annealing		62	10 seconds
Extension		72	20 seconds
Extension	1	72	5 minutes
	1	4	hold

E. **Analysis and Results** *(pages 14-18)*

Using BiOptic *Qsep* series Bio-Fragment Analyzers to analyze the PCR amplicon.

	ALDH2 WT 504Glu	*ALDH2* MT 504Lys	Common band
Target size	119 bp	98 bp	176 bp

The results and interpretation are as below:

Genotype and data		
Wild type: 504 Glu/504 Glu (119, 176 bp)	Mutant type: 504 Lys/504 Lys (98, 176 bp)	Hetero type: 504 Lys/504 Glu (98, 119, 176 bp)