CRISmono™ NRAS Quick Guide

Preparation for Assay

- Before starting, spin down all reagents briefly to collect components clinging under the lid and keep them on ice until ready for use.
- In the case of frozen reagents, they are fully thawed and mixed using a vortex mixer.
- Briefly spin down and keep on ice until ready for use.
- Using a filter tip during all experimental processes is recommended.

Step 1. IVC (Total volume : 10 µL, each)

1-1) Prepare a reaction mix by adding the reagents in the order indicated in the following table to a clean PCR tube:

Reagent	Volume per Sample
● Remov RXN buffer	4 µL
● Stabilizer	1 µL
● NRAS Enzyme mix	4 µL
Genomic DNA (20–150 ng)	1 µL to each tube
	Total Volume 10 µL

1-2) Mix each reagent, then perform vortexing and spin down.

1-3) Place the PCR tube in the pre-set thermal cycler under the conditions shown in the table below and run the program. (Lid temperature: 60 °C)

Step description	Temperature	Time
In vitro Cleavage (IVC)	45 °C	60 min

1-4) After the 60 min reaction, add ● 1 µL of 10X STOP buffer to each reaction mixture and mix by pipetting or vortex.

Step 2. Target amplification (Total volume : 50 µL, each)

2-1) Prepare a reaction mix by adding the reagents listed in the following table to a new PCR tube:

Reagent	Volume per Sample
● NRAS Primer mix	5 µL
○ 2X PCR Master mix	25 µL
Nuclease-free Water	18 µL
IVC product	2 µL
	Total Volume 50 µL

2-2) Mix each reagent, then perform vortexing and spin down.

2-3) Place the PCR tube in the pre-set thermal cycler under the conditions shown in the table below and run the program. (Lid temperature: 105 °C)

Step description	Temperature	Time	Cycles
Pre-denaturation	98 °C	3 min	1
Denaturation	98 °C	10 sec	42
Annealing	55 °C	40 sec	
Extension	72 °C	30 sec	
Final Extension	72 °C	5 min	
Hold	4 °C	∞	1

Step 3. DNA Purification (Clean up)

3-1) Purify DNA from the reaction mixture.

: Column purification, magnetic beads size selection and enzyme purification are possible to use

Step 4. Sanger sequencing

4-1) Please refer to the manufacturer's instructions and recommendations.

Reagent
● NRAS Seq Primer F or NRAS Seq Primer R (10 pmole/µL)

Step 5. Data analysis

5-1) Sequencing chromatogram analysis for NRAS codon 12 and 13(CDS 34-39) sites.

: ATGACTGAGTACAAACTGGTGGTGGTTGGAGCA**GGTGGT**-GTTGGGAAAAGCGCACTGACAATCCAGCTAATC

5-2) VAF (%) is determined by the ratio of peak heights and is considered positive when the VAF value exceeds 10%.

$$\text{VAF (\%)} = \frac{\text{Mutant height}}{\text{Wild-type height + Mutant height}} \times 100$$

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