NucleoGene
PROBE SNP ASSAY
Instructions for Use

Release Date— 01.12.2023

Store at -20°C

REF

NGOT009 300 rxn

For research use only.
Not suitable for diagnostic use.
For professional use only.

Shipping: Shipped at gel ice and dry ice.

Storage Conditions: Store at -20 °C

Additional Storage Conditions: Stock solutions in TE buffer should be aliquoted and stored at -20 °C

Shelf Life: 12 months

Applications:

Single nucleotide polymorphisms (SNPs) are a common source of genetic variation within genomic DNA and typically confer a single nucleotide difference within a specific gene locus, resulting in two alleles of the gene. SNPs within the coding or regulatory regions of a gene can affect function, or more commonly serve as biological markers for inheritance of specific loci. NucleoGene PROBE SNP ASSAY is specially designed to help you genotype and detect your target SNPs in a smooth, specific and sensitive manner.

Description

NucleoGene Biotechnology offers comprehensive collections of prede-signed, preformulated primer-probe sets that help researchers perform SNP studies on a variety of species.

NucleoGene Probe SNP Assays – Target SNP from a variety of species, including human, mouse, rat, Arabidopsis, C. elegans, and Drosophila.

NucleoGene Probe SNP Assays use TaqMan® 5′-nuclease chemistry for amplifying and detecting specific polymorphisms in purified genomic DNA samples. Each assay allows genotyping of individuals for a single nucletide polymorphism (SNP). All assays are developed using our bioinformatics assay design processes that apply heuristic rules that are deduced from both manufacturing and assay performance data.

Each NucleoGene Probe SNP Assays contains:
• Sequence specific forward and reverse primers to amplify the polymophic sequence of interest
• Two TaqMan® minor groove binder (MGB) probes with nonfluorescent quenchers(NFQ):
– One FAM™-labeled probe to detect Allele 1 sequence
– One HEX™-labeled probe to detect Allele 2 sequence
MGB probes at the 3' end bind to the DNA helix minor groove, improving hybridization based assays by stabilizing the MGB probe-template com-plex. This increased binding stability allows the use of probes as short as 13 bases for superior allelic discrimination and assay design flexibility. All MGB probes also include a nonfluorescent quencher (NFQ) that virtually eliminates background fluorescence and provides excellent signal-to-noise ratio for superior assay sensitivity.

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Reporter dye information for the Probe SNP represented in the assay context sequence. The context sequence is the nucleotide sequence su-rounding the SNP site and is provided in the (+) genome strand orientation relative to the NCBI reference genome. The SNP alleles are included in brackets, where the order of the alleles corresponds to the association with probe reporter dyes, where [Allele 1 = FAM™ dye / Allele 2 = HEX™ dye]. If the context sequence is ...XXXXX[A/B]XXXXX...:
• A allele always represents the FAM™ dye
• B allele always represents the HEX™ dye

Required Materials

- PCR reagents
- Real-time PCR instrument
- Reaction plates and accessories for your real-time PCR
- Centrifuge (with plate adapter)
- Disposable gloves
- Microcentrifuge
- Pipette tips, aerosol-resistant
- Pipettors (positive/air-displacement or multichannel)
- Polypropylene tubes (various sizes)
- Vortexer
- Nuclease free water

Prepare the Reaction

1. Thaw on ice, completely resuspend by gently vortexing, then briefly centrifuge to bring liquid to the bottom of the tube:
·NucleoGene Probe SNP Assays (4✕)

2. Mix the master mix reagent by gently swirling the bottle

Calculate the number of reactions that you need for each assay.

Nucleogene Biotechnology recommends performing three replicates of each reaction. Be sure to include on each plate:
·NucleoGene Probe SNP Assays for each DNA sample
· Endogenous control assays
· (Optional) No template controls (NTCs) for each SNP assay on the plate

Prepare the PCR Reaction Mix

1. Thaw on ice, completely resuspend by gently vortexing, then briefly centrifuge to bring liquid to the bottom of the tube:
·NucleoGene Probe SNP Assays (4✕)
· DNA samples(1-20 ng)
2. Mix the master mix reagent by gently swirling the bottle
Calculate the number of reactions that you need for each assay.
Nucleogene Biotechnology recommends performing three replicates of each reaction. Be sure to include on each plate:
·NucleoGene Probe SNP Assays for each cDNA sample
· Endogenous control assays
· (Optional) No template controls (NTCs) for each snp probe assay on the plate

PCR reaction mix component	Volume per 20-µL reaction (µL)	
	Single reaction	Three replicates#
4✕ NucleoGene Probe SNP Assay (Oligo Mix)	5	20
2✕ NucleoGene qPCR Probe Master Mix	10	40
DNA template (1 to 20 ng)	4	16
RNase-free water	1	4

 # Replicate volumes include 20% excess to compensate for volume loss from pipetting.

2. Cap the tube and invert it several times to mix the reaction components.
3. Centrifuge the tube briefly.

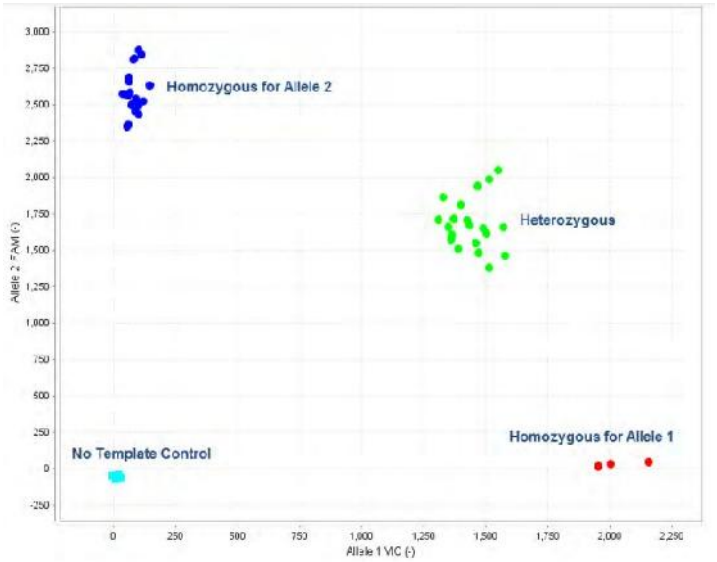
Load the plate

1. Transfer 20 µL of PCR reaction mix into each well of a 48-, 96-, or 384-well reaction plate.
2. Seal the plate with the appropriate cover.
3. Centrifuge the plate briefly.
4. Load the plate into the instrument.

Real Time PCR Protocol

Initialdenaturation	1 cycle	95ºC 15 min
Denaturation	40 cycles	95ºC 15s
Annealing		60ºC 60s*

***Reading is taken from FAM and HEX Channel.**



Typical allelic discrimination plot

Fluorescence signals	Sample genotype
FAM™ signal	Homozygosity for Allele 1
HEX™ signal	Homozygosity for Allele 2
FAM™ and HEX™ signals	Heterozygosity Allele 1-Allele 2

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