SUGENOMICS Biotechnology

BiSNP Genotyping Assay

Catalog No	Size
• BiSNPGA3	1000 Reaction

INSTRUCTIONS FOR USE

1. PRODUCT NAME:
BiSNP Genotyping Assay
Cat No : BiSNPGA3
Size : 1000 reactions

2. MANUFACTURER:
SuGenomik Biyoteknoloji, METU Technopolis, Yenimahalle, Ankara.

3. INTENDED USE
BiSNP Genotyping Assay utilizes a unique form of competitive allele-specific PCR combined with a novel, homogeneous, fluorescence-based reporting system for the identification and measurement of genetic variation occurring at the nucleotide level to detect single nucleotide polymorphisms (SNPs) or inserts and deletions (InDels). BiSNP Genotyping is suitable for use on a variety of equipment platforms and provides flexibility in terms of the number of SNPs and the number of samples able to be analyzed.

4. KIT CONTENT

Content	1000 Reaction
BiSNP Genotyping Assay Mix (sl_50282)	5 X 1000 µl
BiSNP Genotyping Primer Mix (sl_50282)	1 X 150 µl

5. STORAGE CONDITIONS
Store at -20±5°C away from light for 12 months.

6. SPECIMEN PREPARATION AND STORAGE
- Perform DNA isolation using a DNA isolation kit suitable for the samples.
- Pay attention to high DNA purity, as the quality of the isolated DNA will affect the result of allele discrimination.
- Store the isolated DNA samples at +4℃ for a short time and -20℃ for a long time.

7. PRE-TEST PREPARATIONS
- Reagents shall be stored at –20℃ and be avoided from repeated freezing and thawing.
- Reagents shall be used after completely thawed and well mixed.
- This kit was validated on ABI StepOne Plus system and compatible with standard RT-PCR machines such as ABI 7000, 7300,7500, Roche 480, MX3000P, MX3005P, Rotorgene TM6000, Icycler IQTM4/5, Bio-Rad CFX96 and LongGene Q2000B.

8. End-Point REAL-TIME PCR
8.1) Real-Time PCR Solution Preparation
a) Thaw kit components at room temperature.
b) Mix thoroughly to ensure homogeneity, centrifuge briefly and then put on the ice.
c) Prepare the reaction mix based on the table below.
d) It is recommended to use positive and negative controls for each test.
e) Spin down all reaction mixture and move to the qRT-PCR step.

Components	Sample Well	Negative Template Control (NTC) Well
BiSNP Genotyping Assay Mix (sl_50282)	5 µl	5 µl
BiSNP Genotyping Primer Mix (sl_50282)	0,14 µl	0,14 µl
Sample DNA	5 µl	-
Negative Control	-	5 µl
TOTAL Volume	10,14 µl	10,14 µl

Important Notes: It is recommended to run 24 or more samples in a single run.
If a small number of samples are studied, allele discrimination may not be distinguished.
Please add one or two NTC samples to each run.

8.2) Real-Time PCR Set Up Conditions
- Set up the channels of the real-time PCR device according to the table below.

Genes	Channels (Dyes)
Allel1	FAM
Allel2	HEX

Note: Select ROX as the passive reference dye.

- Set up real-time PCR temperature, time, and cycling conditions according to the table below.

BiSNP PCR Reaction Cycling Conditions					
	Stage Name	**Temp.**	**Time**	**Cycle**	**Read**
1	Holding Stage (Pre-PCR Read)	30℃	1 min	1	Yes
2	Holding Stage	95℃	15 min	1	No
3	Cycling Stage (Touchdown)	95℃	20 sec	10	No
		61℃ (Auto Delta Temp: -0.60, Starting Cycle:2)	1 min		
4	Cycling Stage	95℃	20 sec	26	No
		55℃	1 min		
5	Cycling Stage	95℃	20 sec	9	No
		57℃	1 min		
6	Holding Stage (Post-PCR Read)	30℃	1 min	1	Yes

9. Allelic Discrimination Analysis
Perform allele discrimination through the KlusterCaller software (LGC Biosearch Technologies).

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