



## BiSNP Genotyping Assay

**Catalog No** \_\_\_\_\_  
**• BiSNPGA2**      **Size** \_\_\_\_\_  
**100 Reaction**

### INSTRUCTIONS FOR USE

#### 1. PRODUCT NAME:

BiSNP Genotyping Assay

**Cat No** : BiSNPGA2

**Size** : 100 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	100 Reaction
Multiplex Master Mix	500 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°C for a short time and -20°C for a long time.

#### 7. PRE-TEST PREPARATIONS

- > Reagents shall be stored at -20°C 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	5 µl	5 µl
Sample DNA	5 µl	-
Negative Control	-	5 µl
<b>TOTAL Volume</b>	<b>10 µl</b>	<b>10 µl</b>

**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°C	1 min	1	Yes	
2 Holding Stage	95°C	15 min	1	No	
3 Cycling Stage (Touchdown)	95°C	20 sec	10	No	
	61°C (Auto Delta Temp: -0.60, Starting Cycle:2)				
4 Cycling Stage	95°C	20 sec	26	No	
	55°C				
5 Cycling Stage	95°C	20 sec	12	No	
	57°C				
6 Holding Stage (Post-PCR Read)	30°C	1 min	1	Yes	

#### 9. Allelic Discrimination Analysis

Perform allele discrimination through the KlusterCaller software (LGC Biosearch Technologies).