Instructions For Use

Version: 1.0 *Ref: 355096* *Revision date: 2024-12-04*

BRCA1/2 Sanger Sequencing Primer Plate

NimaGen.

Innovators in DNA Sequencing Technologies

Product and Company Information

***BRCA1/2* Sanger Sequencing Primer Plate: Complete Primer Plate for *BRCA1/2* Sanger Sequencing, 80 amplicons x 50 rxn**

REF 355096

Research Use Only

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Symbols Used on Product Labels and in Instructions For Use

Symbol	Description
	Manufacturer
	Use-by date
LOT	Lot number
REF	Reference number
	Temperature limit for storage
Σ	Contains sufficient for <*n*> tests
	GS1 DataMatrix, containing information about the product

Product Description

The *BRCA1/2* Sanger Sequencing Primer Plate is a complete Primer Set for *BRCA1/2* Sanger Sequencing with 80 amplicons. The plate contains enough primer sets for 50 samples. Use the plate for setting up PCR reactions, followed by cycle sequencing and capillary electrophoresis, to screen for mutations in the complete coding region including ±50 bp up- and downstream of each coding exon of the *BRCA1* and *BRCA2* genes.

All primer sets have been optimized in concentration and design to be compatible with the following chemistry for amplification:

- Q5® High-Fidelity 2X Master Mix from New England Biolabs

All primers have been 5' extended with universal M13 tails:

Forward primers with -21M13 and reverse primers with M13Rev to enable cycle sequencing with universal primers.

Universal primers to use in the cycle sequencing reaction are:

M13 Forward primer (-21M13): 5' TGTAAAACGACGGCCAGT 3'

M13 Reverse primer (M13Rev): 5' CAGGAAACAGCTATGACC 3'

Box Content and Storage

The box holds a primer plate containing 80 primer pairs in optimized concentrations, covering both *BRCA* genes.

Description	Reference	Storage
80 primer pairs for 50 reactions each (80 x 2 oligos per well)	355096	Store the plate at -20 to -80 °C

Safety precautions Primer Pairs

Adhere to good laboratory practice.

Check the MSDS safety sheet for complete information.

Required Materials, Not Included

Description	Vendor
Q5® High-Fidelity 2X Master Mix or Other Hot Start Hi-Fi Master Mix	New England Biolabs Multiple Vendors
Pipettes, multichannel (1-10 µL and 20-200 µL) and single 1-10, 1-20, 10-200, 100-1000 microliter)	Multiple Vendors
PCR Plate	Multiple Vendors
Thermal cycler	Multiple Vendors
General disposables (tubes, vials, filter tips)	Multiple Vendors
Mini Plate Spinner	Multiple Vendors
Adhesive PCR seals	Multiple Vendors
Molecular Biology Grade Water	Multiple Vendors

Protocol

Guidelines for Genomic DNA quality

The performance of the primers in the plates for *BRCA1* and *BRCA2* sequencing is highly dependent on the quality and quantity of the used genomic DNA. NimaGen strongly recommends checking quality and quantity of your DNA sample by A260/A280 ratio analysis. Follow the recommended quantity of DNA in the original PCR setup (25-50 ng/µL). Too much input DNA will result in possible inhibition and/or overloaded signals.

Setting up the PCR reactions

1. For 1 DNA sample, prepare the following PCR Mix in a 2 mL vial:

A. PCR Mix

PCR Master Mix (2x)	880 µL
Human Genomic DNA (20 – 50 ng/µL)	90 µL
Water (molecular biology grade)	790 µL
TOTAL	***1760 µL***

2. Dispense 80 x 18 µL of the PCR (A) mix in a new PCR plate in columns 1 – 10.
3. Transfer 2 µL of each primer pair from the *BRCA1/2* Sanger Sequencing Primer Plate to the wells of columns 1-10 of the previous PCR plate to make a total volume of 20 µL. Figure 1 shows the location of each primer in the *BRCA1/2* Sanger Sequencing Primer Plate.

Figure 1:

	1	2	3	4	5	6	7	8	9	10	11	12
A	*BRCA1* exon 1	*BRCA1* exon 10	*BRCA1* exon 11/8	*BRCA1* exon 15	*BRCA1* exon 23	*BRCA2* exon 8	*BRCA2* exon 11/3	*BRCA2* exon 11/11	*BRCA2* exon 13	*BRCA2* exon 20		
B	*BRCA1* exon 2	*BRCA1* exon 11/1	*BRCA1* exon 11/9	*BRCA1* exon 16	*BRCA1* exon 24	*BRCA2* exon 9	*BRCA2* exon 11/4	*BRCA2* exon 11/12	*BRCA2* exon 14/1	*BRCA2* exon 21		
C	*BRCA1* exon 3	*BRCA1* exon 11/2	*BRCA1* exon 11/10	*BRCA1* exon 17	*BRCA2* exon 1	*BRCA2* exon 10/1	*BRCA2* exon 11/5	*BRCA2* exon 11/13	*BRCA2* exon 14/2	*BRCA2* exon 22		
D	*BRCA1* exon 4	*BRCA1* exon 11/3	*BRCA1* exon 11/11	*BRCA1* exon 18	*BRCA2* exon 2	*BRCA2* exon 10/2	*BRCA2* exon 11/6	*BRCA2* exon 11/14	*BRCA2* exon 15	*BRCA2* exon 23&24		
E	*BRCA1* exon 5	*BRCA1* exon 11/4	*BRCA1* exon 11/12	*BRCA1* exon 19	*BRCA2* exon 3	*BRCA2* exon 10/3	*BRCA2* exon 11/7	*BRCA2* exon 11/15	*BRCA2* exon 16	*BRCA2* exon 25		
F	*BRCA1* exon 6	*BRCA1* exon 11/5	*BRCA1* exon 12	*BRCA1* exon 20	*BRCA2* exon 4	*BRCA2* exon 10/4	*BRCA2* exon 11/8	*BRCA2* exon 11/16	*BRCA2* exon 17	*BRCA2* exon 26		
G	*BRCA1* exon 7	*BRCA1* exon 11/6	*BRCA1* exon 13	*BRCA1* exon 21	*BRCA2* exon 5&6	*BRCA2* exon 11/1	*BRCA2* exon 11/9	*BRCA2* exon 11/17	*BRCA2* exon 18	*BRCA2* exon 27/1		
H	*BRCA1* exon 8	*BRCA1* exon 11/7	*BRCA1* exon 14	*BRCA1* exon 22	*BRCA2* exon 7	*BRCA2* exon 11/2	*BRCA2* exon 11/10	*BRCA2* exon 12	*BRCA2* exon 19	*BRCA2* exon 27/2		

4. Seal the plate with adhesive (PCR) seal, then vortex and spin the plate briefly.

Note: make sure to tightly seal all wells to prevent evaporation during PCR.

5. Run the reactions in the thermal cycler:

Temp	Duration	Cycles
95 °C	10 minutes	HOLD
95 °C	15 seconds	32 x
60 °C	30 minutes	
72 °C	1 minute	
72 °C	7 minute	HOLD
4 °C	∞	HOLD

Post-PCR Processing

When the PCR product is ready, it can be stored at -20 to -80 ^{0}C until further processing or continue straight away. The PCR product can be purified for Sanger Sequencing using the AmpliClean or the ExS-Pure kits, followed by the BrilliantDye™ Terminator v3.1, or v.1.1, Cycle Sequencing Kit, followed by a purification with D-Pure™ DyeTerminator Cleanup Kit.

Customer Support

For technical assistance, please contact us at techsupport@nimagen.com.

Revision History

Section	Summary of changes	Version	Date
All	New document.	1.0	2024-12-04

Legal Notice

Disclaimer

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