AmoyDx® HRD Focus Panel

Instructions for Use

REF 8.06.0047 20 tests/kit For Illumina NextSeq 550Dx

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Background

The Homologous Recombination Repair (HRR) pathway plays an important role in the repair of double-strand breaks, which is a major cause of cancer development. It has been demonstrated that loss of function of HRR genes, Homologous Recombination Deficiency (HRD) is the inability to repair double strand breaks and will cause a higher risk of developing cancer[1-5]. Patients with HRD showed higher responses to poly ADP ribose polymerase inhibitors (PARPi) and platinum-containing therapies[6-7]. HRD Score testing and *BRCA1/2* mutations testing have been approved for patient selection for PARPi therapy.

Intended Use

The AmoyDx® HRD Focus Panel is a next-generation sequencing (NGS) based *in vitro* diagnostic assay intended for qualitative determination of homologous recombination deficiency (HRD) status via detection and classification of single nucleotide variants (SNVs) and insertions and deletions (InDels) in protein coding regions and intron/exon boundaries of the *BRCA1* and *BRCA2* genes and the determination of the Genomic Scare Score (GSS) which is an algorithmic measurement of genomic instability status, using DNA isolated from formalin-fixed paraffin embedded (FFPE) tumour tissue specimens. The assay is intended to aid in identifying ovarian cancer patients with positive HRD status.

This assay is not automated and is for laboratory professional use only.

Principles of the Procedure

The test kit is based on the Halo-shape ANnealing and Defer-Ligation Enrichment (HANDLE) system technology to capture the target gene region (Figure 1). The unique molecular identifier (UID) is introduced to both ends of each DNA fragment to trace back to original template for error correction. The library construction time of HANDLE system is 5 hours with 1 hour hands-on time. After quality control (QC), the prepared DNA libraries are sequenced on Illumina sequencing platforms to detect the *BRCA1/2* gene mutations and HRD status.

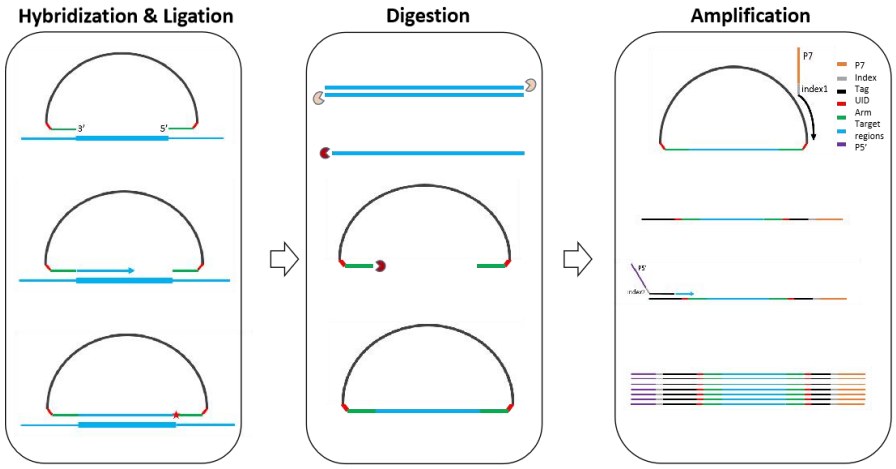

Figure 1 Principle of library construction (HANDLE system)

The probe contains an extension arm and a ligation arm which are complementary to the target gene region. First, the extension arm and ligation arm are anchored to the target gene region, and the DNA is extended from the extension arm to the ligation arm using DNA polymerase. Next, the nicks are connected with the ligase to generate the circular products. The remaining linear probes, single-strand and double-strand nucleic acid are digested with the exonuclease. Finally, the universal PCR amplification is performed to enrich the target

libraries.

Kit Contents

This kit contains the following components in Table 1.

Table 1 Kit Contents

Serial No.	Components	Main Ingredient	Quantity
1	HRD-Probe	Oligonucleotides	23 μL/tube ×1
2	HRD-Hybridization Buffer	Tris-HCl, K^+, Mg^{2+}	23 μL/tube ×1
3	HRD-Extension Ligation Master Mix	DNA polymerase, dNTPs, DNA Ligase, Ligation buffer	23 μL/tube ×1
4	HRD-Exonuclease A	DNA Exonuclease	35 μL/tube ×1
5	HRD-Exonuclease B	DNA Exonuclease	23 μL/tube ×1
6	HRD-PCR Master Mix	Tris, Mg^{2+}, dNTPs, DNA polymerase	550 μL/tube ×1
7	HRD-N7 Primer *	Oligonucleotides	5 μL/tube ×12
8	HRD-S5 Primer *	Oligonucleotides	5 μL/tube ×8
9	HRD-Positive Control A	DNA	36 μL/tube ×1

* For labeling and sequence information of the primers, refer to Appendix I.

Storage and Stability

The kit requires shipment on frozen ice packs and the shipping time should be less than one week. All contents of the kit should be stored immediately upon receipt at -20℃±5℃.

The shelf-life of the kit is twelve months. The maximal number of freeze-thaw cycles is five.

Materials Required but Not Supplied

1) DNA quantification kit: QuantiFluor dsDNA System (Promega) or Qubit® dsDNA HS Assay Kit (Thermo Fisher Scientific) is recommended.
2) Fluorometer: Quantus™ Fluorometer (Promega) or Qubit 2.0/3.0/4 Fluorometer (Thermo Fisher Scientific) is recommended.
3) DNA extraction kit: AmoyDx® Magnetic FFPE DNA Extraction Kit (Amoy Diagnostics) or MagPure FFPE DNA LQ Kit (Magen Biotech) is recommended. It is recommended to use RNase A to degrade RNA during the DNA extraction.
4) DNA purification kit: Agencourt AMPure XP Kit (Beckman Coulter Genomics) or CleanNGS magnetic beads (Vdobiotech) is recommended.
5) Capillary electrophoresis analyzer and related kit: Agilent 2100 Bioanalyzer System and Agilent DNA 1000 Kit (Agilent Technologies) are recommended.
6) Thermocycler: Applied Biosystems™ 2720 Thermal Cycler is recommended.
7) Illumina PhiX Control v3 (Illumina) is recommended.
8) Sequencing reagent: Illumina 300 cycles (Paired-End Reads, 2×150 cycles) is recommended.
9) Sequencer: Illumina platform NextSeq 550Dx is recommended.
10) Magnetic stand: DynaMag™-2 Magnet (Thermo Fisher Scientific) is recommended.

11) Mini centrifuge.

12) Vortex mixer.

13) Ice box for 0.2 mL and 1.5 mL tubes.

14) 1.5 mL nuclease-free centrifuge tubes.

15) 0.2 mL nuclease-free PCR tubes.

16) Nuclease-free filtered pipette tips.

17) Absolute ethanol (AR).

18) Nuclease-free water.

19) TE-low solution (10 mM Tris, 0.1 mM EDTA, pH 8.0).

Precautions and Handling Requirements

For *in vitro* diagnostic use.

Precautions

- Please read the instruction carefully and become familiar with all components of the kit prior to use, Strictly follow the instruction during operation.
- DO NOT use the kit or any kit component after their expiry date.
- DO NOT use any other reagent from different lots in the test.
- DO NOT use any other reagent from another test kit.

Safety Information

- Handle all specimens and components of the kit as potentially infectious material using safe laboratory procedures.
- Only trained professionals can use this kit. Please wear suitable lab coat and disposable gloves while handling the reagents.
- Avoid contact of skin, eyes and mucous membranes with the chemicals. In case of contact, flush with water immediately.
- DO NOT pipet by mouth.

Decontamination and Disposal

- The kit contains positive control; strictly distinguish the positive control from other reagents to avoid contamination which may cause false positive results.
- PCR amplification is extremely sensitive to cross-contamination. The flow of tubes, racks, pipets and other materials used should be from pre-amplification to post-amplification, and never backwards.
- Gloves should be worn and changed frequently when handling samples and reagents to prevent contamination.
- Use separate, dedicated pipettes and filtered pipette tips when handling samples and reagents to prevent exogenous nucleic acid contamination to the reagents.
- All disposable materials are for one-time use. DO NOT reuse.
- Unused reagents, used kit, and waste must be disposed properly.

Cleaning

- After the experiment, wipe down the work area, spray down the pipettes and equipment with 75% ethanol or 10% hypochlorous acid solution.

Specimen Preparation

- Sample DNA should be extracted from FFPE tissue sample of ovarian cancer patients.
- The FFPE tissue sample should be fixed in 10% neutral buffered formalin for not more than 24 hours (recommend within 6 hours). It is recommended that the tumor cell content is not less than 30%. For samples with a tumor cell content less than 30%, we recommend to re-collect the samples or use the microdissection technology to collect tumor cells. It's recommended to use the central section of paraffin blocks. The freshly cut sections of FFPE tissue should be used for DNA extraction immediately. The recommended storage time for FFPE tissue samples is within 12 months.
- It's recommended to use a commercialized extraction kit to perform FFPE DNA extraction and use RNase A to degrade RNA during the DNA extraction. After the extraction, measure the concentration of extracted DNA using Quantus™ or Qubit® Fluorometer, the DNA concentration should be more than 6.25 ng/μL, and the total DNA should be more than 50 ng.
- The extracted DNA should be used for DNA library preparation immediately, if not, it should be stored at -20℃±5℃ for no more than 12 months, avoid repeated freezing and thawing.

Assay Procedure

Note:

- *It is recommended to include a HRD-Positive Control A (PC) in the process of library preparation, sequencing, data analysis.*
- *When using the kit for the first time, or when necessary, it is recommended to use a no template control (NTC) to verify the absence of contamination. The NTC could be used for the quality control of the library construction process, and no need to run the sequencing or data analysis process.*
- *During the following DNA library preparation process, please use the corresponding adaptor (ie. 96-well tray) in the thermocycler to avoid the PCR products evaporation.*

1. Pre-denaturation

1) Take out the **DNA samples, HRD-Positive Control A** and **TE-low solution** and thaw the reagents at room temperature. When the reagents are completely thawed, vortex each tube for 5~10 seconds and centrifuge briefly, then put them on the ice.
2) Prepare the pre-denaturation solution in a 0.2 mL PCR tube according to Table 2.

Table 2 Pre-denaturation solution

Reagent	Volume
TE-low solution	8-χ μL
DNA	χ μL
Total	**8 μL**

Note:

- *For FFPE samples, "χ" stands for the volume of 50~100 ng DNA (100 ng is recommended).*
- *For Positive Control, the DNA concentration of HRD-Positive Control A is 6 ng/μL, take 8 μL to construct library (χ=8). The mutation result of PC is listed in table of Appendix II.*
- *For NTC, use the TE-low solution of 8 μL, χ=0.*
- *If the sample is degraded severely, try to increase the amount of DNA up to 200 ng.*

3) Mix the solution thoroughly by vortexing, and centrifuge briefly. Incubate in a thermocycler, set the reaction volume as 8 μL and perform the following program: 98℃ for 5 min, then put the tubes on the ice immediately.

2. Hybridization

1) Take out the **HRD-Probe** and **HRD-Hybridization Buffer** and thaw the reagents at room temperature. When the reagents are completely thawed, vortex each tube for 5~10 seconds and centrifuge briefly, then put them on the ice.

2) Assemble the hybridization reaction mix on ice according to Table 3.

Table 3 Hybridization reaction solution

Reagent	Volume
Pre-denaturation product	8 μL
HRD-Probe	1 μL
HRD-Hybridization Buffer	1 μL
Total	**10 μL**

Note:

- *It is recommended to pre-mix the HRD-Probe and HRD-Hybridization Buffer together when there are more than 3 samples.*

3) Mix the solution thoroughly by vortexing, and centrifuge briefly. Incubate in a thermocycler, set the reaction volume as 10 μL and perform the following program: 95℃ for 5 min, 60℃ for 2 hours, hold at 4℃.

***Note**:*

- ***Important!** The hybridization at 60℃ should be a strictly 2 hour hybridization, otherwise the risk of quality control failure may be increased.*
- *Keep the tubes at low temperature after hybridization is finished, as high temperature in the room may increase the non-specificity. It is recommended to put the ice box beside the thermocycler in advance, when hybridization step is finished, take out the reaction tube and put it in the ice box immediately.*
- *The hybridization products should be stored at 2~8℃ for no more than 20 hours if not proceeding to the next step.*

3. Extension-Ligation

1) Take out the **HRD-Extension Ligation Master Mix** and thaw the reagent at room temperature. When the reagents are completely thawed, vortex each tube for 5~10 seconds and centrifuge briefly, then put them on the ice.

2) Take out the above PCR tubes from the thermocycler and put them on the ice. Add 1 μL **HRD-Extension Ligation Master Mix** into the PCR tubes, mix thoroughly by vortexing, and centrifuge briefly. Incubate in a thermocycler, set the reaction volume as 11 μL and

perform the following program: 60℃ for 10 min, hold at 4℃.

***Note**:*

- *It is recommended to put in the reaction tube when the thermocycler is heated to above 50℃ after starting the PCR program.*
- *Keep the tubes at low temperature after extension-ligation is finished, as high room temperature may increase the non-specificity. It is recommended to put the ice box beside the thermocycler in advance, when it is finished, take out the reaction tube and put it in ice box immediately.*
- *Perform the subsequent exonuclease digestion step immediately when the extension-ligation step is finished.*

4. Exonuclease Digestion

1) Take out the **HRD-Exonuclease A** and **HRD-Exonuclease B**, vortex each tube for 5~10 seconds and centrifuge briefly, then put them on the ice.

2) Assemble the exonuclease digestion reaction mix on ice according to Table 4.

Table 4 Exonuclease digestion reaction solution

Reagent	Volume
Extension-Ligation product	11 μL
HRD-Exonuclease A	1.5 μL
HRD-Exonuclease B	1 μL
Total	**13.5 μL**

3) Mix the solution thoroughly by vortexing, and centrifuge briefly. Put the tubes in a thermocycler, set the reaction volume as 14 μL and run the following program: 37℃ for 30 min, 95℃ for 10 min, hold at 4℃.

***Note**: The products of exonuclease digestion should be stored at 2~8℃ for no more than 20 hours if not proceeding to the next step.*

5. PCR Amplification

1) Take out the **HRD-N7 Primer, HRD-S5 Primer** and **HRD-PCR Master Mix** and thaw the reagents at room temperature. When the reagents are completely thawed, vortex each tube for 5~10 seconds and centrifuge briefly, then put them on the ice.

2) Assemble the PCR reaction mix on ice according to Table 5.

Table 5 PCR reaction solution

Reagent	Volume
Exonuclease digestion product	13.5 μL
HRD-PCR Master Mix	25 μL
Nuclease-free water	8.5 μL
HRD-N7 Primer	1.5 μL
HRD-S5 Primer	1.5 μL
Total	**50 μL**

***Note**:*

- *Each of the HRD-N7 Primer or HRD-S5 Primer has a different index sequence. Use a combination of HRD-N7 Primer and HRD-S5 Primer for each library, and different libraries should use different combination if to be sequenced in the same sequencing run. The detailed information for the index sequence is shown in Appendix I.*

- *Transfer the prepared tubes to the amplification room to perform PCR amplification and the following purification to avoid contamination.*

3) Mix the solution thoroughly by vortexing, and centrifuge briefly. Put the tubes in a thermocycler, set the reaction volume as 50 μL, then perform the following program according to Table 6.

Table 6 PCR Program

Temperature	Time	Cycles
98℃	30 s	1
98℃	10 s	21
61℃	30 s	
72℃	20 s	
72℃	5 min	1
4℃	∞	1

***Note**:*

- *The PCR products should be stored at 2~8℃ for no more than 20 hours if not proceeding to the next step.*

6. Purification

1) Take out the AMPure XP beads or CleanNGS magnetic beads and equilibrate them to room temperature for 30 min, and vortex the bottle of the beads to resuspend any magnetic particles that may have settled.
2) Add 37 μL beads and 40 μL PCR products into 1.5 mL centrifuge tubes, mix thoroughly by vortexing or pipetting up and down 10~20 times, then incubate the mixture at room temperature for 5 min.
3) Place the centrifuge tubes onto the magnetic stand for 3~5 min until the solution turns clear. Gently remove and discard the supernatant while the centrifuge tube is on the magnetic stand. Do not touch the beads with pipette tip.
4) Keep the tubes on the magnetic stand, add 200 μL fresh 80% ethanol into the centrifuge tube, and keep it at room temperature for at least 30 seconds. Remove and discard the supernatant while the tube is on the magnetic stand.
5) Repeat the above step 4).
6) Air dry the beads for 2~3 min while the tube is on the magnetic stand with the lid open. Do not over-dry the beads; this may result in lower recovery of DNA. Elute the samples when the beads are still dark brown and glossy looking, but when all visible liquid has evaporated. When the beads turn lighter brown and start to crack they are too dry.
7) Remove the centrifuge tubes away from the magnetic stand, add 20 μL TE-low solution, mix thoroughly by vortexing. Then incubate the mixture at room temperature for 3 min.
8) Place the tube onto the magnetic stand for 3~5 min until the solution turns clear. Transfer the supernatant into a new 1.5 mL centrifuge tube to obtain the DNA library. Do not touch the beads with pipette tip.

***Note**: It is recommended to store the library at -20℃±5℃ for no more than one week if not proceed directly to sequencing.*

7. DNA Library Quality Control (QC)

1) DNA library concentration QC: Quantify the DNA library concentration using Quantus™ or Qubit® Fluorometer, the DNA

concentration should be no less than 20 ng/μL.

2) DNA fragment QC(optional): Quantify the DNA library fragment size using Agilent 2100 Bioanalyzer System and Agilent DNA 1000 Kit, the main peak of the DNA fragment size should be at 230~330 bp, as shown in Figure 2.

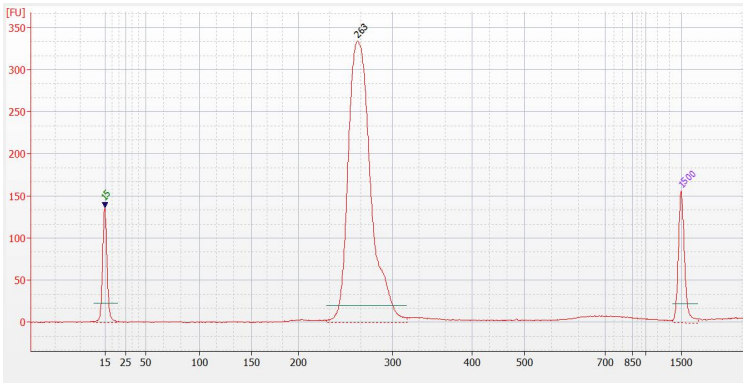

Figure 2 Diagram of the quality control of the library on Agilent 2100 Bioanalyzer DNA1000 Chip Reagent

Note:

- *If the library concentration is less than 20 ng/μL, the original DNA may be of poor quality, the DNA concentration may be inaccurate or there may be operational errors during the experiment. The DNA concentration should be retested and the DNA libraries should be re-constructed. For poor DNA quality sample, up to 200 ng of DNA input is recommended.*
- *If the NTC library detects the target length fragment (230-330 bp), there may be contamination during the experiment and the experiment should be repeated.*

8. Sequencing:

Illumina NextSeq 550Dx and the matched 300 cycles (Paired-End Reads, 2×150 cycles) reagents are recommended for the sequencing and the recommended number of samples/run on the Mid and High-output flow cell is summarized in Table 7. In the event of insufficient sample number in a sequencing run, it is mandatory to add additional libraries of PC sequencing samples as a supplement, or to increase the percentage of spiked-in PhiX. It is recommended to sequence each HRD Focus sample from minimum 4 Gb to a maximum of 6 Gb.

Table 7 Recommended Sequencer and Sample Quantity per Run

Sequencer	Flow Cell	Read Length	Sample Number/run	Spike in PhiX, %	Remark
NextSeq 550Dx	Mid-output (40 Gb/run)	2×150 bp	8 samples + 1PC	1% (0.4 Gb)	Maximum sample number per run on mid-output flow cell
	High-output (120 Gb/run)	2×150 bp	28 samples + 1PC	1% (0.4 Gb)	Maximum sample number per run on high-output flow cell

Note:

In the event of under-sequencing and over sequencing, raw data being generated from per run per sample is lesser than 4 Gb or more than 6 Gb per sample, re-sequencing is mandatory to ensure stability of HRD focus analysis, response from BRCA and SNP uniformity or effective depth. PhiX Spike in percentage can be adjusted accordingly from 1% up to maximum 50% to avoid under or over

sequencing matter (as per illustration in Table 8).

Table 8 Guidance on PhiX Spiking

Sequencer	Flow Cell	Read Length	Sample Number/run	Spike in PhiX, %	Remark
NextSeq 550Dx	Mid-output (40 Gb/run)	2×150 bp	4 samples + 1PC	50% (20 Gb)	Suggested minimum sample number per run for Mid and High-output flow cells. User should validate internally on the PhiX spiking for sample number/ run outside of the recommendation stated on Table 7.
	High-output (120 Gb/run)	2×150 bp	14 samples + 1PC	50% (60 Gb)	

For *more HRD focus related queries, please kindly contact AmoyDx technical team or local channel partner.*

Perform the denaturation and dilution of the libraries according to the instrument's instructions. The final concentration of sequencing library is recommended in Table 9.

Table 9 Recommended Final Concentration of Sequencing Library

Illumina Sequencer	Final Concentration
NextSeq 550Dx	0.6~0.8 pM

Note: *The concentration converting formula:*

$$Library\ \ Concentration\ [nM]\ =\ \frac{Library\ Concentration\ [ng/\mu L]\times 10^{6}}{660\times 268}$$

9. Data Analysis:

When the sequencing is finished, adopt AmoyDx NGS Data Analysis System (ANDAS) Data Analyzer to analyze the sequencing data and detect the *BRCA1/2* gene mutations and HRD status. Select the module ADXHS-tHRD-GSS for FFPE sample or HRD-Positive Control A data analysis.

Check Q30 value for the sequencing run:

If Q30 value of the sequencing run is ≥ 75%, the sequencing run is qualified. If not, the sequencing run is unqualified.

If Q30 value of each library is ≥ 75%, the library sequencing data is qualified. If not, the library sequencing data is unqualified.

Quality control:

The qualified criteria for data QC is shown in Table 10.

Table 10 Quality control of data analysis

QC parameter	QC standard (FFPE sample or HRD-Positive Control A)
Q30	≥ 75%
Coverage	≥ 98%
BRCA_Uniformity (BRCA region)	≥ 95%
BRCA_effectiveDepth (BRCA region)	≥ 400×
SNP_Uniformity (SNPs region)	≥ 90%
SNP_effectiveDepth (SNPs region)	≥ 200×

Note:

Q30: one base call in 1,000 is predicted to be incorrect meaning a base call accuracy of 99,9%.

Coverage: The proportion of the sequencing data mapped region to the designed target region.

BRCA_Uniformity (BRCA region): The uniformity of BRCA1 and BRCA2 gene regions, defines the proportion of target areas where the depth is greater than 20% of the average depth.

BRCA_effectiveDepth (BRCA region): The average of the depth of all the individual base of BRCA1 and BRCA2 gene regions, after strand-specific base calibrate.

SNP_Uniformity (SNPs region): The uniformity of the SNPs regions, defines the proportion of target areas where the depth is greater than 20% of the average depth.

SNP_effectiveDepth (SNPs region): The average of the depth of all the individual base of SNPs region, after strand-specific base calibrate.

Result Interpretation:

- For SNV/InDel mutations of FFPE samples, the mutations are detected if meeting the following requirements.

  The effective depth is ≥ 100×, the mutant allele frequency is ≥ 3%, the depth of mutatant allele (ADP) of non (C>T, G>A) and non-polymer mutation is ≥ 6, the ADP of C>T, G>A and polymer mutation is ≥ 8.

  Note: Polymer here means the regions with 5 or more consecutive identical nucleotides.

- For Genomic Scar Score (GSS) of FFPE samples

  The cut-off of GSS is 50.0.

- For HRD Status results of FFPE samples

  A positive HRD status result is defined by either the presence of a pathogenic/likely pathogenic variant in *BRCA1* and/or *BRCA2* genes or a positive GSS (GSS ≥ 50.0).

Note:

The analysis result of the HRD-Positive Control A should be positive as shown in Appendix II, otherwise, the HRD testing is unqualified, it's necessary to check if there is any operational error during the experiment and retest the samples.

According to the classification standards of the International Agency of Research on Cancer (IARC) and the American College of Medical Genetics (ACMG), the BRCA1/2 variants can be divided into 5 classes: pathogenic variant (5), likely pathogenic variant (4), variant of uncertain significance (VUS) (3), likely benign variant (2), benign variant (1). Only pathogenic (5) or likely pathogenic (4) variant is defined as BRCA positive.

Performances

1) Limit of Detection (LoD)

The LoD for SNV/InDel detection is 5% allele frequency at 50 ng DNA input, and the LoD of the GSS portion of the assay is at 30% tumor content.

2) Accuracy

The positive percent agreement (PPA) of SNV/InDel of BRCA1/2 gene was 100%， and the consistency between BRCA1/2 deficient and GSS positive was more than 90%.

Note: *Tumors selected as BRCA1/2 deficient had either:*

(i) One pathogenic/likely pathogenic mutation in BRCA1 or BRCA2, with LOH in the wild-type copy;

(ii) Two pathogenic/likely pathogenic mutations in the same gene;

(iii) Promoter methylation of BRCA1 with LOH in the wild-type copy.

3) Specificity

The negative percent agreement (NPA) of SNV/InDel of BRCA1/2 gene was 100%, and the GSS negative concordance was 100%.

4) Precision

The overall analytical concordance of the BRCA1/2 mutation status and GSS status across 6 replicates (using 2 lots of reagents, 2 different operators and across multiple days) was 100%.

5) Interfering substance

The overall analytical concordance of the BRCA1/2 mutation status and GSS status was 100% by the presence of potential interfering substances (formalin at a final concentration of 0.1%, xylene at 35 mmol/L, ethanol at 21.7 mmol/L, and proteinase K at 0.08 mg/mL) , when compared samples without additional interfering substance. These results demonstrate that the assay are minimally impacted or not impacted by the presence of any of the substances tested in this study.

Limitations

1) The kit is to be used only by personnel specially trained in the techniques of PCR and NGS.
2) The results can be used to assist clinical diagnosis, combining with other clinical and laboratory findings.
3) The kit has been only validated for use with human FFPE samples.
4) This test identifies germline and somatic variants in the tumor but does not distinguish between the two.
5) Reliable results are dependent on proper sample processing, transport, and storage.
6) Different parts of the tumor tissue or different sampling times may lead to different mutation results due to tumor heterogeneity.
7) A Negative result can not completely exclude the existence of gene variants. Low tumor cell content, severe DNA degradation or the frequency under the limit of detection may also cause a false negative result.
8) InDels ≤ 26 bp in length can be correctly detected by this kit. InDels larger than 26 bp has not been fully validated, the detection ability may decrease as the length of the InDels increases.
9) When InDel occurs on primers of two consecutive probes, it may cause a false negative result.
10) The test results of this kit are for clinical reference only and should not be used as the sole basis for individualized treatment of patients. Clinicians should make comprehensive judgments on the test results based on factors such as the patient's condition, drug indications, treatment response and other laboratory test indicators.

References

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Symbols

EC REP	Authorized Representative in the European Community	IVD	In Vitro Diagnostic Medical Device
	Manufacturer	REF	Catalogue Number
LOT	Batch Code		Use By
Σ	Contains Sufficient for <n> Tests		Temperature Limitation
	Consult Instructions For Use		Keep Dry
	This Way Up		Fragile, Handle With Care

Appendix I

Index Sequence Information for Primers

Name	Primer Index Information	Illumina Nextera XT v2 Set B No.
HRD-N716	TAGCGAGT	N716
HRD-N718	GTAGCTCC	N718
HRD-N719	TACTACGC	N719
HRD-N720	AGGCTCCG	N720
HRD-N721	GCAGCGTA	N721
HRD-N722	CTGCGCAT	N722
HRD-N723	GAGCGCTA	N723
HRD-N724	CGCTCAGT	N724
HRD-N726	GTCTTAGG	N726
HRD-N727	ACTGATCG	N727
HRD-N728	TAGCTGCA	N728
HRD-N729	GACGTCGA	N729

Name	Primer Index Information	Illumina Nextera XT v2 Set B No.
HRD-S502	CTCTCTAT	S502
HRD-S503	TATCCTCT	S503
HRD-S505	GTAAGGAG	S505
HRD-S506	ACTGCATA	S506
HRD-S507	AAGGAGTA	S507
HRD-S508	CTAAGCCT	S508
HRD-S510	CGTCTAAT	S510
HRD-S511	TCTCTCCG	S511

Appendix II

Pathogenic and Likely pathogenic Mutation and GSS status in HRD-Positive Control A

No	Gene	BRCA1/2 mutation status and GSS status	Expected Allele Frequency
1	*BRCA1*	NM_007294:exon20:c.5251C>T:p.(R1751*)	65%
2	*BRCA2*	NM_000059:exon11:c.4777G>T:p.(E1593*)	20%
3	/	GSS status: GSS positive	/

*Class of variant: according to the classification criteria from American College of Medical Genetics and Genomics (ACMG).