AmoyDx
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AmoyDx® BRCA Pro Panel

Instructions for Use

REF 8.06.0104 24 tests/kit For Illumina NextSeq 500, NextSeq 550Dx, MiSeqDx

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Background

BRCA1 and *BRCA2* are tumor suppressor genes, which are involved in DNA damage repair and transcriptional regulation by homologous recombination. Type I poly ADP-ribose polymerase (PARP-1) is a modified enzyme that plays an important role in DNA damage repair and apoptosis. Tumor cells with mutations in the *BRCA1* gene and the *BRCA2* gene often rely on PARP-1 to repair DNA damage. Therefore, PARP inhibitors can inhibit the repair of DNA damage in tumor cells by inhibiting the function of PARP-1. Clinical studies have shown that multiple tumors with *BRCA1* or *BRCA2* mutations can benefit from the treatment of PARP inhibitors [1-3]. Currently, the US FDA has approved PARP (poly ADP-ribose polymerase) inhibitors for the treatment of ovarian cancer and breast cancer patients with *BRCA1* gene or *BRCA2* gene mutation. The *BRCA1* gene and *BRCA2* gene are located on human chromosomes 17 and 13 respectively, each containing 22 and 26 exon regions with coding functions. According to current clinical studies and databases, mutation types include insertions, deletions, and point mutation. Mutations affecting gene function are up to more than 3,000, distributing in various functional areas of genes, and there is no hotspot mutation region. These mutations can be classified into five categories according to the classification criteria of the International Agency for Research on Cancer (IARC) and the American College of Medical Genetics (ACMG) (Appendix II), with Class 4 and 5 being effective populations to PARP inhibitors.

Intended Use

The AmoyDx® BRCA Pro Panel is a next-generation sequencing (NGS) based *in vitro* diagnostic assay intended for qualitative detection and classification of single nucleotide variants (SNVs), insertions and deletions (InDels), and large rearrangements (LRs) in protein coding regions, intron/exon boundaries, some introns and UTR regions of the *BRCA1* and *BRCA2* genes, using DNA isolated from peripheral whole blood or formalin-fixed paraffin embedded (FFPE) tumour tissue specimens. The detection of large rearrangement deletions and duplications is available for blood-derived DNA only. The assay is intended to aid in identifying breast cancer or ovarian cancer patients with pathogenic, or likely pathogenic BRCA variants.

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 which is an improved Molecular Inversion Probe (MIP) technology to capture the target gene region (Figure 1). During the library construction process, each individual DNA molecule is tagged with a unique molecular index (UMI) at both ends, which allows high sensitivity in variant detection by eliminating any library amplification and sequencing bias. The test kit uses DNA extracted from tissue or blood samples, and it offers a time saving protocol that can be completed within 5 hours, and requires just about 1 hour of hands-on time.

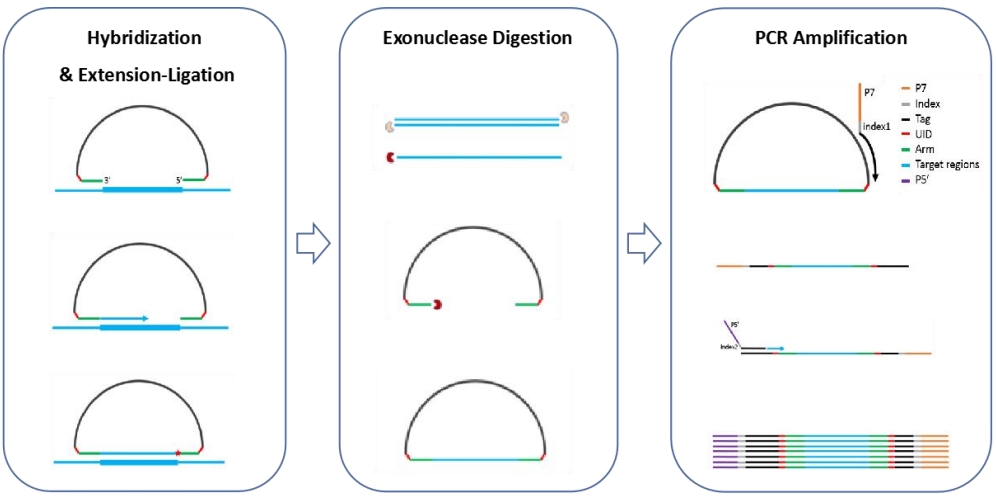

Figure 1 Principle of library construction (HANDLE system)

The probe contains an extension arm and a ligation arm which are complementary to the target gene region. Firstly, the probe anneals onto the DNA template of the target region. Secondly, the DNA is extended from the extension arm to the ligation arm with the help of DNA polymerase, then the nicks are repaired to generate the circular products with the help of DNA ligase. Next, the remaining linear probes, single-strand and double-strand DNA are digested with the help of enzyme exonuclease, and only the target circular DNA will be kept for PCR amplification. Finally, the universal PCR amplification is performed to enrich the target DNA, and the magnetic bead-based purification

is performed to obtain the final library.

After quality control (QC), the qualified libraries could be sequenced on Illumina sequencing platform. The sequencing data can be analyzed by AmoyDx NGS data analysis system (ANDAS) to detect the genomic variants in the target region.

Kit Contents

This kit contains the following components in Table 1.

Table 1 Kit contents

Serial No.	Components	Main Ingredient	Quantity
1	HS-Probe	Oligonucleotides	28 μL/tube ×1
2	HS-Hybridization Buffer	Tris-HCl, K^+, Mg^{2+}	28 μL/tube ×1
3	HS-Extension Ligation Master Mix	DNA polymerase, dNTPs, DNA Ligase, Ligation buffer	28 μL/tube ×1
4	HS-Exonuclease A	DNA Exonuclease	40 μL/tube ×1
5	HS-Exonuclease B	DNA Exonuclease	28 μL/tube ×1
6	HS-PCR Master Mix	dNTPs, DNA polymerase,PCR buffer	600 μL/tube ×1
7	HS-S5 Primer *	Oligonucleotides	5 μL/tube ×8
8	HS-N7 Primer *	Oligonucleotides	5 μL/tube ×12
9	HS-Positive Control	DNA	60 μL/tube ×1
10	HS-Negative Control	DNA	60 μL/tube ×1

* For labeling and sequence information of the primers, refer to Appendix Table S1.

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 recommended maximum freeze-thaw cycle is five cycles.

Additional Reagents and Equipment Required but Not Supplied

1) PCR instrument: Applied Biosystems™ 2720 Thermal Cycler, MiniAmp™ Thermal Cycler or Bio-Rad T100™ Thermal Cycler is recommended.
2) DNA quantification kit: QuantiFluor dsDNA System (Promega) or Qubit® dsDNA HS Assay Kit (Thermo Fisher Scientific) is recommended.
3) Fluorometer: Quantus™ Fluorometer (Promega) or Qubit® 2.0/3.0/4 Fluorometer (Thermo Fisher Scientific) is recommended.
4) DNA extraction kit: AmoyDx® Blood/Bone Marrow DNA Kit (Amoy Diagnostics) or QIAamp DSP DNA Blood Mini Kit (Qiagen) is recommended for DNA extraction from whole blood sample; AmoyDx® FFPE DNA Kit (Amoy Diagnostics) or MagPure FFPE DNA LQ Kit (Magentec) is recommended for DNA extraction from FFPE tissue sample. It is recommended to use RNase A to degrade RNA during the FFPE DNA extraction.
5) DNA purification kit: Agencourt AMPure XP Kit (Beckman Coulter) or CleanNGS magnetic beads (Vdobiotech) is recommended.
6) Capillary electrophoresis analyzer and related kit: Agilent 2100 Bioanalyzer system and Agilent DNA 1000 Reagents (Agilent Technologies, Cat. No. 5067-1504) or Agilent High Sensitivity DNA Kit (Agilent Technologies, Cat. No. 5067-4626), or E-Gel™ Power Snap Electrophoresis System (Thermo Fisher Scientific, Cat. No. G8300) and E-Gel™ EX Agarose Gels, 2% (Thermo Fisher Scientific, Cat. No. G4010-02) are recommended.
7) Sequencing Instrument: Illumina NextSeq 500/NextSeq 550Dx/MiSeqDx is recommended.
8) Sequencing reagent: Illumina 300 cycles (Paired-End Reads, 2×150 cycles) is recommended.
9) Illumina PhiX Control V3.
10) Magnetic stand: DynaMag™-2 Magnet (Thermo Fisher Scientific, Cat. No. 12321D) is recommended.
11) Mini centrifuge.
12) Vortex mixer.

13) Ice box for 0.2 mL and 1.5 mL tubes.
14) Nuclease-free 1.5 mL centrifuge tubes.
15) Nuclease-free 0.2 mL PCR tubes.
16) Nuclease-free filtered pipette tips.
17) Absolute ethanol (AR).
18) PCR-grade water (nuclease-free).
19) TE-low solution (10 mM Tris, 0.1 mM EDTA, pH 8.0) or 10 mM Tris solution (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, and strictly follow the instruction during operation.
- DO NOT use the kit or any kit component after their expiry date.
- DO NOT use any other reagents from different lots in the tests.
- DO NOT use any other reagents in the other test kits.

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 skin, eyes and mucous membranes contact 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.
- The 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 peripheral whole blood or FFPE tissue samples of ovarian cancer or breast cancer patients.
- The FFPE tissue sample should be fixed by 10% neutral buffered formalin for 6~24 hours (no more than 24 hours). The freshly cut sections of FFPE tissue should be used for DNA extraction immediately. The storage time for the FFPE tissue should be less than 12 months.
- It is recommended that the tumor cell content is no less than 20%.
- The peripheral whole blood should be more than 2 mL. The EDTA anticoagulant is recommended during the blood collection, avoid using heparin anticoagulant. The blood sample should be extracted immediately or transported at 2~8℃, if not, store the blood sample at -20±5℃ for no more than 3 years.
- It is recommended to use a commercialized DNA extraction kit to perform the DNA extraction according to the sample type. And it is recommended to use RNase A (Thermo Fisher Scientific, Cat. No. EN0531) to degrade RNA during the FFPE DNA extraction. After

DNA extraction, measure the concentration of extracted DNA using Quantus™ or Qubit® Fluorometer. The DNA concentration should be more than 3.75 ng/μL, and total DNA should be more than 30 ng. For unqualified samples, re-collection or re-extraction are required.

- The qualified 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 HS-Positive Control (PC) and a HS-Negative Control (NC) in the process of library preparation, sequencing, and data analysis.*
- *During the following DNA library preparation process, please use the corresponding adapter in the PCR instrument to avoid PCR product evaporation.*
- *It is recommended to use fluorescent dye method (Quantus™ or Qubit® Fluorometer) for all the DNA concentration measurement steps.*

1. Pre-denaturation

1.1. Take out the **DNA samples** and **TE-low solution** (not provided) and thaw the reagents at room temperature. When the reagents are completely thawed, mix well on a vortex mixer and centrifuge briefly, then keep the tube on ice.

1.2. Assemble the pre-denaturation reaction on ice by adding the following components according to Table 2.

Table 2. Pre-denaturation reaction

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

Note:

- *For blood samples, "χ" stands for the volume of 30~50 ng DNA (50 ng is recommended).*
- *For FFPE tissue samples, "χ" stands for the volume of 30~100 ng DNA (50 ng is recommended).*
- *For PC or NC, the DNA concentration is 6.25 ng/μL, take 8 μL to construct library (χ=8).*

1.3. Mix the solution thoroughly by vortexing or pipetting, and centrifuge briefly, then place the sample in a thermocycler. Set the reaction volume as 8 μL and perform the following program: 98℃ for 5 min, then put the tubes on ice immediately.

2. Hybridization

2.1. Take out the **HS-Probe** and **HS-Hybridization Buffer** and thaw the reagent at room temperature. When the reagents are completely thawed, mix well on a vortex mixer and centrifuge briefly, then keep the tube on ice.

2.2. Take out the above pre-denaturation product from the thermocycler and keep the tube on ice. Assemble the hybridization reaction on ice by adding the following components according to Table 3.

Table 3. Hybridization reaction

Reagent	Volume
Pre-denaturation product (from step 1.3)	8 μL
HS-Probe	1 μL
HS-Hybridization Buffer	1 μL
Total	**10 μL**

***Note**: It is recommended to prepare **freshly ready-to-use premix** of HS-Probe and HS-Hybridization Buffer for precise pipetting when perform three or more samples simultaneously.*

2.3. Mix the solution thoroughly by vortexing or pipetting, and centrifuge briefly, then place the sample in a thermocycler. Set the reaction volume as 10 μL and perform the following program: 95℃ for 5 min, 60℃ for 2 h, 4℃ hold.

***Note**:*

- *Keep the tubes at low temperature after hybridization is finished, as high temperature like room temperature may increase the*

non-specificity. It is recommended to place the ice box besides the thermocycler, and when it is finished, take out the reaction tube and put it in ice box immediately.

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

3. Extension-Ligation

3.1. Take out the **HS-Extension Ligation Master Mix** and thaw the reagent on ice. When the reagents are completely thawed, mix well on a vortex mixer and centrifuge briefly, then keep the tube on ice.

3.2. Take out the above hybridization product from the thermocycler and keep the tube on ice. Add 1 µL **HS-Extension Ligation Master Mix** into the PCR tube, mix the solution thoroughly by vortexing or pipetting, and centrifuge briefly, then place the sample in a thermocycler, set the reaction volume as 11 µL and perform the following program: 60°C for 10 min, 4°C hold.

***Note**:*

- *Perform the subsequent exonuclease digestion step immediately when the extension-ligation step is finished.*

4. Exonuclease Digestion

4.1. Take out the **HS-Exonuclease A** and **HS-Exonuclease B**, mix well on a vortex mixer and centrifuge briefly, then keep the tube on ice.

4.2. Assemble the exonuclease digestion reaction on ice by adding the following components according to Table 4.

Table 4. Exonuclease digestion reaction

Reagent	Volume
Extension-Ligation product (from step 3.2)	11 µL
HS-Exonuclease A	1.5 µL
HS-Exonuclease B	1 µL
Total	**13.5 µL**

4.3. Mix the solution thoroughly by vortexing or pipetting, and centrifuge briefly, then place the sample in a thermocycler, set the reaction volume as 14 µL and perform the following program: 37°C for 30 min, 95°C for 10 min, 4°C hold.

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

5. PCR Amplification

5.1. Take out the **HS-S5 Primer, HS-N7 Primer** and **HS-PCR Master Mix** and thaw the reagents at room temperature. When the reagents are completely thawed, mix well on a vortex mixer and centrifuge briefly, then keep the tube on ice.

5.2. Assemble the PCR amplification reaction on ice by adding the following components according to Table 5.

Table 5 PCR amplification reaction

Reagent	Volume
Exonuclease digestion product (from step 4.3)	13.5 µL
HS-PCR Master Mix	25 µL
PCR-grade water (nuclease-free)	8.5 µL
HS-S5 Primer	1.5 µL
HS-N7 Primer	1.5 µL
Total	**50 µL**

***Note**:*

- *Each of the HS-S5 Primer or HS-N7 Primer has a different index sequence. Use different combination of HS-S5 Primer and HS-N7 Primer for each sample library. **Do not** use the same combination of index for two or more sample libraries in one sequencing run. The detailed information for the index sequence is shown in Appendix Table S1.*
- *Transfer the prepared tubes to the amplification room to perform PCR amplification and the following purification to avoid contamination.*

5.3. Mix the solution in each PCR tube thoroughly by vortexing or pipetting, and centrifuge briefly, then place the sample in a thermocycler, set the reaction volume as 50 µL, and then perform the following program according to Table 6.

Table 6. PCR program

Temperature	Time	Cycles
98℃	1 min	1
98℃	20 s	25
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 proceed to the next step.*

6. Purification

6.1. 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.

6.2. Add **37 μL beads** and **40 μL PCR products** into a clean 1.5 mL centrifuge tube, mix thoroughly by vortexing or pipetting, then incubate for 5 min at room temperature.

6.3. 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.

6.4. Keep the tubes on the magnetic stand, add 200 μL of freshly prepared 80% ethanol to the tube. Incubate at room temperature for at least 30 seconds, and then carefully remove and discard the supernatant.

6.5. Repeat step 6.4 once.

6.6. Briefly spin the tube, and put the tube back in the magnetic stand. Completely remove the residual ethanol, and air dry the beads for 2~3 min while the tube is on the magnetic stand with the lid open.

***Note**: Do not over-dry the beads. This may result in lower recovery of DNA target.*

6.7. Remove the tube from the magnet. Elute DNA target from the beads by adding 30 μL TE-low solution (pH 8.0) or 10 mM Tris solution (pH 8.0) (not provided), mix thoroughly by vortexing or pipetting, and incubate for 3 min at room temperature.

6.8. Put the tube in the magnetic stand for 3~5 min until the solution turns clear. Without disturbing the bead pellet, transfer the supernatant into a clean 1.5 mL centrifuge tube to obtain the final library.

***Note**: The purified library should be stored at -20±5 ℃ for no more than two weeks if not proceed directly to sequencing.*

7. Library Quality Control (QC)

7.1. Library concentration QC: Quantify the DNA library concentration using Qubit Fluorometer or QuantiFluor, for tissue DNA library, the concentration should be no less than 5 ng/μL; for whole blood DNA library, the concentration should be no less than 10 ng/μL.

7.2. Library fragment size QC (optional) : Assess the library quality on an Agilent Bioanalyzer DNA chip or other fragment size analysis instrument, the main peak size of the DNA fragment should be at 250~400 bp, as shown in Figure 2.

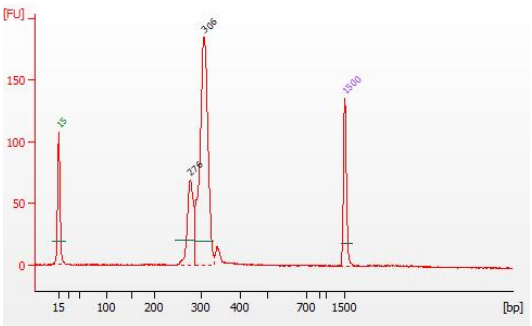

Figure 2. Example of the qualified library distribution on Agilent 2100 Bioanalyzer (Agilent DNA 1000 Reagents)

Note:

- *If the library QC pass, then move to sequencing. If not, the library should be reconstructed.*

- *If the library concentration QC fails (tissue DNA library concentration is less than 5 ng/μL, or whole blood DNA library concentration is less than 10 ng/μL), the library is unqualified. Please first check whether it is caused by operating errors and try to repeat the test. If the operating error has been ruled out, it may be caused by the poor quality of the sample DNA, and it is recommended to extend the hybridization time and perform overnight hybridization (60℃ for 12~18 h) to rebuild the library, or re-collect and re-test the sample.*

8. Sequencing

Illumina 300 cycles (Paired-End Reads, 2×150 cycles) and the matched reagents and instruments are recommended for the sequencing. The recommended percentage of Illumina PhiX Control v3 is 1%. For blood sample, the sequencing data per sample should be no less than 60 Mb; for FFPE tissue sample, the sequencing data per sample should be no less than 300 Mb.

The suggested sample quantity per run is listed in Table 7.

Table 7. Recommended sequencing instruments and sample quantity per run

Sequencer	Flow Cell	Read Length	For Germline Variants (60 Mb/sample)	For Somatic Variants (300 Mb/sample)
MiSeqDx	V3	2×150bp	up to 96#	~25
NextSeq 500/NextSeq 550Dx	Mid/High	2×150bp	up to 96#	up to 96#

 # Maximum 96 indexes available. The PhiX percentage must be adjusted to ensure over-sequencing is not performed.

Perform the denaturation and dilution of the libraries according to the instrument's instructions. The final concentration of sequencing library is recommended in Table 8.

Table 8. Recommended final concentration of sequencing library

Sequencing Instrument	Final Concentration
MiSeqDx	6~8 pM
NextSeq 500/NextSeq 550Dx	0.6~0.8 pM

Note:

- *If different types of libraries need to load in the same flow cell, the loading concentration need to be adjusted appropriately according to the experience of the laboratory.*
- *The concentration converting formula:*

$$Library\ Concentration\ [nM] = \frac{Library\ Concentration\ [ng/\mu L] \times 10^6}{660 \times 300}$$

9. Data Analysis

When the sequencing is finished, adopt AmoyDx ANDAS Data Analyzer to analyze the sequencing data.

Check Q30 value for the sequencing data:

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

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

Select the analysis module:

If the Q30 value is qualified, select the appropriate analysis module according to the sample type and sensitivity parameter, as shown in Table 9.

Table 9. Analysis modules for different samples

Sample Type	Data Volume Requirements	Analysis Module	Alterations Detected
Blood	60 Mb/sample	ADXHS-gBRCA-CNV	SNVs, InDels, LRs
FFPE tissue	300 Mb/sample	ADXHS-tBRCA	SNVs, InDels

Check QC parameters of the analyzing data:

- For blood samples, the minAmp (minimum depth) should be no less than 50×.
- For FFPE tissue samples, the effectiveDepth (average effective depth) should be no less than 400×.

Result Interpretation:

The mutations are detected if meeting the following requirements.

- For blood samples

  The minAmp (minimum depth) should be no less than 50×, the mutant allele frequency should be no less than 20%.

- For FFPE tissue samples

  The filter thresholds are listed in Table 10.

Table 10. Filter thresholds

Analysis Module	Depth	Filter Thresholds for Non-Polymer and Non-STR Variants		Filter Threshold for Polymer and STR Variants
		Filter Threshold for Non (C>T, G>A) Variants	Filter Threshold for C>T or G>A Variants	
ADXHS-tBRCA	≥50×	1%	2%	2%

***Note**:*

- *Q30: One base call in 1,000 is predicted to be incorrect meaning a base call accuracy of 99.9%.*
- *effectiveDepth: The average depth of the target region after UMI calibration.*
- *Depth: The effective depth of the detected variant.*
- *Polymer means the regions with 5 or more consecutive identical nucleotides.*
- *Short tandem repeats (STRs) means short tandemly repeated DNA sequences that involve a repetitive unit of 2 or more base pairs, with 4 or more repeats.*
- *The PC should be detected as positive results for the corresponding variants as shown in Table S2, and the NC should be detected as negative (no pathogenic or likely pathogenic variants). Otherwise, the testing is unqualified, it is necessary to check if there is any operational error and the sample should be retested.*
- *According to the classification standards of the International Agency of Research on Cancer (IARC) and the American College of Medical Genetics (ACMG), based on the population data, computational and predictive data, functional data, segregation data and other evidence data, 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.*

Performance

1) Limit of Detection (LoD)

The LoD of SNV/InDel was 2% allele frequency at non-polymer region, and 5% allele frequency at polymer and STR regions.

2) Accuracy

Studies of whole blood samples demonstrated 100% positive percent agreement (PPA), 100% negative percent agreement (NPA), and 100% overall percent agreement (OPA) for SNV/InDel, and 96% PPA, 100% NPA and 99.6% OPA for LR.

Studies of clinical FFPE samples demonstrated 100% PPA, 100% NPA and 100% OPA for SNV/InDel.

3) Precision

Repeatability studies of whole blood samples demonstrated 100% PPA and 100% NPA for all variants (SNV/InDel and LR) assessed across lots of reagents, operators, and days.

Repeatability studies of clinical FFPE samples demonstrated 100% PPA and 100% NPA for all variants (SNV/InDel) assessed across lots of reagents, operators, and days.

4) Inteference impact

Interference studies of whole blood samples demonstrated 100% PPA and 100% NPA for all variants (SNV/InDel and LR) by the presence of potential interfering substances (Hemoglobin at a final concentration of 2 g/L, Triglyceride at 37 mM), when compared samples without additional interferents.

Interference studies of clinical FFPE samples demonstrated 100% PPA and 100% NPA for all variants (SNV/InDel) by the presence of potential interfering substances (xylene at a final concentration of 35 mmol/L, ethanol at 21.7 mmol/L, and proteinase K at 0.08 mg/mL), when compared samples without additional interferents. 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 kit has been only validated for use with human peripheral whole blood samples and FFPE tissue samples.
3) Negative results 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.
4) A negative results of large rearrangement can not completely exclude the existence of large rearrangement amplification and deletion of *BRCA1* and *BRCA2*, the gain/loss exon regions that are too small may cause a false negative result.
5) The exon 2 sequence of the *BRCA1* gene contains complex secondary structure, high AT content and pseudogenes, may cause a false positive or false negative result for large rearrangement detection.
6) False negative results may occur when the deletion occurs on two adjacent probes of two consecutive amplicons.
7) Different parts of the tumor tissue or different sampling times may cause different mutation results due to tumor heterogeneity.
8) Reliable results are dependent on proper sample processing, transport, and storage.
9) 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

1. Kaufman B, Shapira-Frommer R, Schmutzler RK, et al. Olaparib monotherapy in patients with advanced cancer and a germline BRCA1/2 mutation. J Clin Oncol 33:244-50, 2015.

2. Mateo J, Carreira S, Sandhu S, et al. DNA-Repair Defects and Olaparib in Metastatic Prostate Cancer. N Engl J Med 373:1697-708, 2015.

3. Oza AM, Cibula D, Benzaquen AO, et al. Olaparib combined with chemotherapy for recurrent platinum-sensitive ovarian cancer: a randomised phase 2 trial. Lancet Oncol 16:87-97, 2015.

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

Table S1. Index Sequence Information for Primers

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

Primer Name	Primer Index Information	Illumina Nextera XT v2 Set D No.
HS-S513	TCGACTAG	S513
HS-S515	TTCTAGCT	S515
HS-S516	CCTAGAGT	S516
HS-S517	GCGTAAGA	S517
HS-S518	CTATTAAG	S518
HS-S520	AAGGCTAT	S520
HS-S521	GAGCCTTA	S521
HS-S522	TTATGCGA	S522

Table S2. Pathogenic or likely pathogenic variants in HS-Positive Control

No.	Gene	CDS Change
1	*BRCA2*	NM_000059.3:exon11:c.3599_3600del:p.(C1200*):p.(Cys1200Ter)
2	*BRCA2*	NM_000059.3:exon11:c.5351del:p.(N1784Tfs*7):p.(Asn1784ThrfsTer7)
3	*BRCA2*	NM_000059.3:exon19:c.8351G>A:p.(R2784Q):p.(Arg2784Gln)
4	*BRCA1*	NM_007294.4:intron20:c.5277+1G>A:p.?:p.?
5	*BRCA1*	NM_007294.4:exon20:c.5266dup:p.(Q1756Pfs*74):p.(Gln1756ProfsTer74)

Note: *Variants in No. 4~5 are germline variants and used for the quality control when using ADXHS-gBRCA-CNV module for data analysis; Variants in No. 1~5 are used for quality control when using ADXHS-tBRCA module for data analysis.*