RingCap®

Human Endometrial Cancer Molecular Classification Detection Kit

High Throughput Sequencing

Instruction for Use

Product Name

Human Endometrial Cancer Molecular Classification Detection Kit (High-Throughput Sequencing)

Packing Specification

16 Tests/kit, 32 Tests/kit

Intended Use

This kit is used to take the nucleic acids extracted from formalin-fixed paraffin-embedded (FFPE) or fresh tissue of cancer patients as the test sample for the qualitative detection of the mutation status of relevant genes, including 28 genes on the DNA level and 50 microsatellite loci (see Appendix Table 2). Additionally, the assay type also includes SNVs, Indels, CNVs and MSI.

Technological Principle

High Throughput Sequencing, also known as Next Generation Sequencing (NGS), can be divided into semiconductor sequencing, DNA nanosphere sequencing and so on according to different sequencing principles. NGS enables the sequencing of up to millions of target nucleic acids at once, provides abundant variation information in short time in a cost-efficient way. Highlighting the characteristics of high output and high resolution, NGS has drawn more and more attention in multiple signaling pathways and target studies of cancer.

This kit is based on ordinary PCR platform, combines specific modified primers and RingCap® loop mediated ligation amplification technique is used to detect mutant genes in nucleic acids samples. Specific modified primers are used to amplify the target sequences by precise PCR. At the same time, RingCap® loop mediated link amplification technology is used to modify the end of the amplified product and connects the specific sequence end. Combined with the use of special PCR reaction program, high specific 5×RingCap Mix, the target sequences in the sample nucleic acids can be constructed on the ordinary PCR platform for high-throughput sequencing, so as to realize the accurate and rapid detection of multi genes and multi-target mutations.

Kit Contents

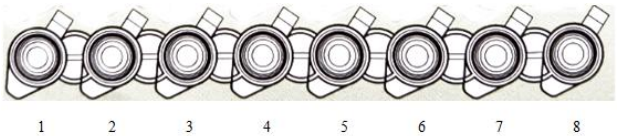
Table 1. Kit Contents

No.	Content Name	Main Content	Strip Color	16 Tests/kit			32 Tests/kit			Note
				Volume	Quantity	8-Tube Strip	Volume	Quantity	8-Tube Strip	
1	**EC-DNA-1 PCR Strip**	Primer, Buffer	Blue	15 μL	16 tubes	2 strips	15 μL	32 tubes	4 strips	Each tube contains same reagent.
2	**EC-DNA-2 PCR Strip**	Primer, Buffer	Pink	15 μL	16 tubes	2 strips	15 μL	32 tubes	4 strips	Each tube contains same reagent.
3	**UDI 1-8 Reaction Strip**	UDI primer, Buffer	Purple	15μL	8 tubes	1 strip	15 μL	8 tubes	1 strip	Each tube represents an UDI.
4	**UDI 9-16 Reaction Strip**	UDI primer, Buffer	Green	15μL	8 tubes	1 strip	15 μL	8 tubes	1 strip	Each tube represents an UDI.
5	**UDI 17-24 Reaction Strip**	UDI primer, Buffer	White	——	——	——	15 μL	8 tubes	1 strip	Each tube represents an UDI.
6	**UDI 25-32 Reaction Strip**	UDI primer, Buffer	Yellow	——	——	——	15 μL	8 tubes	1 strip	Each tube represents an UDI.
7	**5×RingCap Mix**	Enzyme	——	280 μL	1 tube	——	280 μL	2 tubes	——	——
8	**EC Negative Control**	Wild type DNA	——	50 μL	1 tube	——	50 μL	1 tube	——	——
9	**EC Positive Control**	DNA with specific mutation sequence, wild-type DNA	——	50 μL	1 tube	——	50 μL	1 tube	——	——

Note 1: In UDI reaction strips, different UDI numbers respectively contain different UDI recognition sequences (see Appendix Table 1).

Note 2: The reaction solutions had been pre-loaded into 8-Tube strips, as shown in Figure 1.

Figure 1. UDI numbers of 8-Tube Strips



Note 3: The contents of different batches of reagents cannot be mixed.

Additional required Equipment and Materials

1. Nucleic acids extraction kit: Nucleic Acid Extraction Kit (FFPE DNA) (Xiamen Spacegen Co., Ltd, Cat. No. SPG-HSD002R/003R), Nucleic Acid Extraction Kit (FFPE DNA) (Xiamen Spacegen Co., Ltd, Cat. No. SPG-HSD001R) or Nucleic Acid Extraction Kit (Micro DNA) (Xiamen Spacegen Co., Ltd, Cat. No. SPG-HSMD001R/002R)

2. Quantification kit of nucleic acids: Quanti Fluor® dsDNA System (Promega, Cat. No. E2670) or Qubit® dsDNA HS Assay Kit (Thermo Fisher Scientific, Cat. No. Q32851/Q32854), Qubit® ssDNA Assay Kit (Alternatively) (Thermo Fisher Scientific, Cat. No. Q10212)
3. Fluorometer: Quantus™ Fluorometer (Promega, Cat. No. E6150) or Qubit™4.0 Fluorometer (Thermo Fisher Scientific, Cat. No. Q33238)
4. Magnetic beads: SG Pure Beads (Xiamen Spacegen Co., Ltd, Cat. No. SPG-PB001R/002R) or HighPrep™ PCR (MagBio, Cat. No. AC-60005/ AC-60050/ AC-60250/ AC-60500)
5. Sequencing reagents and corollary reagents to be purchased separately: Selecting the corresponding sequencing reagent and related reagents according to the gene sequencer
   (1) Illumina corollary reagents: PhiX Control V3 (Illumina, Cat. No. FC-110-3001)
   (2) MGI corollary reagents: MGIEasy universal library conversion kit (APP-A) (MGI, Cat. No. 1000004155), High throughput sequencing primer kit (App-C) (Alternatively) (MGI, Cat. No. 1000027472), High throughput sequencing primer kit (App-D) (Alternatively) (MGI, Cat. No. 1000028550)
6. Magnetic rack
7. Microvolume UV-visible spectrophotometer
8. Ethanol absolute (Analytical Grade)
9. TE Buffer (pH 8.0)
10. Nuclease-Free Water
11. Nuclease-Free pipette tips with filter

Transportation, Stability and Storage

1. This product is shipped on frozen ice packs. The contents of the shipment should be stored immediately upon receipt at -15℃ to -25℃ in a constant-temperature freezer and protected from light. Repeated thawing and freezing should be avoided. Do not exceed a maximum of 5 freeze-thaw cycles. The shelf-life of the kits is 12 months.
2. Check labels for the production date and expiration date of the kit.

Applicable Instruments

1. Library preparation PCR apparatus: ABI 9700, ABI 2720, ABI Veriti, ABI Mini Amp, etc.
2. Sequencing instruments:
   (1) Illumina sequencing instruments (Miseq, NextSeq 500/550, Miniseq, etc)
   (2) MGI sequencing instruments (MGISEQ-2000, DNBSEQ-G99RS, etc)
   (3) Sikun sequencing instruments (Sikun 2000, Sikun 1000, Sikun 500, Sikun RapidGS480, etc)
   (4) For details on other sequencing instruments, please contact SPACEGEN.

Specimen Material

The quality of the nucleic acids to be detected is critical. Please collect samples according to the following recommended sample types:

1. Recommended sample types: FFPE, fresh tissue.
2. FFPE samples: It is recommended to choose FFPE samples stored within 2 years, which should contain at least 20% of tumor cells after pathological evaluation, and use no less than 8 pieces of 5 μm section or 5 pieces of 10 μm section for nucleic acids extraction.
3. Fresh tissue samples: The diameter of fresh tissue shouldn't be less than 5 mm, the mass shouldn't be less than 20 mg, and ensure that at least 20% of the collected pathological tissue is tumor lesions.

Experimental Procedure

Note: Parallel Library preparation of **EC Positive Control** (EC PC) and **EC Negative Control** (EC NTC) with the tested sample is suggested.

I. Library Enrichment

1. Reagent preparation: Thaw the **EC-DNA-1 PCR Strip** (Blue) and **EC-DNA-2 PCR Strip** (Pink) as needed at room temperature until no ice is present in the tubes, briefly centrifuge the strip before use. Place the **5×RingCap Mix** on ice after centrifugation.

Note: Unused PCR Strip tubes to avoid repeated freeze-thaw.

2. Sample preparation:
   (1) Nucleic acid extraction and quality control: Commercial nucleic acids extraction kit is recommended to extract genomic DNA from the samples. Assess the quality of sample DNA with a Microvolume UV-visible spectrophotometer, the ratio of OD_{260}/OD_{280} should be within the range of 1.8-2.2, quantify sample DNA with a Fluorometer, the concentration should be ≥5 ng/μL, the total

amount of DNA should be ≥50 ng. Once the DNA quality or quantity is not conformed with the above requirements, re-extract DNA with new and/or larger input. DNA is recommended to Library preparation immediately or store at -15℃ to -25℃ for no more than 12 months.

(2) DNA sample: Dilute DNA sample to 5 ng/μL with TE Buffer (pH 8.0) based on the effective DNA concentration measured by the Fluorometer, and the volume ≥10 μL.

3. Add 5μL of **5×RingCap Mix** to **EC-DNA-1 PCR Strip** (Blue) and **EC-DNA-2 PCR Strip** (Pink).
4. Gently remove the cap of the **EC-DNA-1 PCR Strip** (Blue) and **EC-DNA-2 PCR Strip** (Pink), and sequentially add 5 μL of the template into the respective tube, cap the tubes carefully.
5. Vortex and centrifuge the tubes slightly and avoid creating air bubbles.
6. Load the PCR centrifuge tubes above into the thermal cycler, then set up and run the program according to Table 2.

Table 2. PCR Amplification Procedure

Step	Temperature	Time	Cycle Number
Pre-denaturation	98℃	2 minutes	1
Denaturation	98℃	15 seconds	15
Annealing	65℃	4 minutes	
Hold	4℃	∞	1

Note: Proceed to "Purification of Enriching Products", or store the products at 2℃ to 8℃ within 8 hours or at -15℃ to -25℃ within 24 hours. Storing for more than 24 hours is not suggested.

II. Purification of Enriching Products

Note: Transfer the magnetic beads to room temperature and vortex thoroughly to disperse magnetic beads before use. Prepare fresh 70% ethanol with Nuclease-Free Water.

1. Transfer all of PCR enrichment product of **EC-DNA-1 PCR Strip** and **EC-DNA-2 PCR Strip** each to a new 1.5 mL centrifuge tube, add 50 μL (1×sample volume) of magnetic beads to each tube, pipet up and down 5 times to mix magnetic beads suspension thoroughly with the product.
2. Incubate the mixture for 5 minutes at room temperature.
3. Place the tubes on a magnetic rack for 2 minutes until the solution becomes clear, carefully remove and discard the supernatant without disturbing magnetic beads.

Note: The magnetic beads contain amplified library and should not be discarded.

4. Add 150 μL of freshly prepared 70% ethanol to each tube, rotate the tubes clockwise or counterclockwise five times. Place the tubes on the magnetic rack for 2 minutes until the solution becomes clear, carefully remove and discard the supernatant without disturbing magnetic beads.
5. Repeat step 4 one more time for a second wash.
6. Remove all the ethanol from the tubes, and keep the tubes on the magnetic rack for 5 minutes at room temperature to air-dry the magnetic beads (avoid over-dry).
7. Remove the tube from the magnetic rack, add 35 μL of TE Buffer (pH 8.0) to each tube to fully infiltrate the magnetic beads, and vortex thoroughly (or mix by pipetting at least half the total volume up and down at least 5 times), briefly centrifuge to collect the droplets.
8. Incubate the mixture for 5 minutes at room temperature.
9. Place the tubes on the magnetic rack for 2 minutes until the solution becomes clear, carefully transfer and store the supernatant (i.e. purified product), store at -15℃ to -25℃ or proceed to "Library Preparation".

III. Library Preparation

Note: Use different UDI for different samples.

1. Reagent preparation: Thaw the UDI Reaction Strip based on samples amount at room temperature until no ice is present in the tubes, briefly centrifuge the strip before use. Place the **5×RingCap Mix** on ice after centrifugation.
2. Add 5 μL of **5×RingCap Mix** to UDI Reaction Strip.
3. Gently remove the cap of the UDI Reaction Strip, and sequentially add 5 μL of the amplified purified product into the respective tube, cap the tubes carefully.
4. Vortex and centrifuge the tubes slightly and avoid creating air bubbles.

5. Load the UDI Reaction Strip tubes above into the thermal cycler, then set up and run the program according to Table3.

Table 3. PCR Amplification Procedure

Step	Temperature	Time	Cycle Number
Pre-denaturation	95℃	3 minutes	1
Denaturation	95℃	15 seconds	12
Annealing	65℃	30 seconds	
Extension	72℃	30 seconds	
Final Extension	72℃	2 minutes	1
Hold	4℃	∞	1

Note: Proceed to "Library Purification", or store the products at 2℃ to 8℃ within 8 hours or at -15℃ to -25℃ within 24 hours. Storing for more than 24 hours is not suggested.

IV. Library Purification

Note: Transfer the magnetic beads to room temperature and vortex thoroughly to disperse the magnetic beads before use. Prepare fresh 70% ethanol with Nuclease-Free Water.

1. Transfer 25 μL of PCR product each to a new 1.5 mL centrifuge tube, add 25 μL (1×sample volume) of magnetic beads to each tube, pipet up and down 5 times to mix the bead suspension thoroughly with the product.
2. Incubate the mixture for 5 minutes at room temperature.
3. Place the tubes on a magnetic rack for 2 minutes until the solution becomes clear, carefully remove and discard the supernatant without disturbing magnetic beads.

Note: The magnetic beads contain amplified library and should not be discarded.

4. Add 150 μL of freshly prepared 70% ethanol to each tube, rotate the tubes clockwise or counterclockwise five times. Place the tubes on the magnetic rack for 2 minutes until the solution becomes clear, carefully remove and discard the supernatant without disturbing magnetic beads.
5. Repeat step 4 one more time for a second wash.
6. Remove all the ethanol from the tubes, and keep the tubes on the magnetic rack for 5 minutes at room temperature to air-dry the magnetic beads (avoid over-dry).
7. Remove the tubes from the magnetic rack, add 35 μL of TE Buffer (pH 8.0) to each tube to fully infiltrate the magnetic beads, and vortex thoroughly (or mix by pipetting at least half the total volume up and down at least 5 times), briefly centrifuge to collect the droplets.
8. Incubate the mixture for 5 minutes at room temperature.
9. Place the tubes on the magnetic rack for 2 minutes until the solution becomes clear, carefully remove and store the supernatant (i.e. **library**) or store at -15℃ to -25℃ or proceed to next step.

V. Library Quantification

Bioanalyzer is recommended for the quality control of library fragments. For NTC libraries, PC library and all sample DNA libraries, the target fragments should be in 200-350 bp. Quantification kit of nucleic acids is recommended to measure the concentration of sample library and should be more than 0.5 ng/μL.

VI. Library dilution, mixing and sequencing

1. Illumina sequencing platform
   (1) According to the library concentration measured by the Fluorometer, use the following formula to convert the molar concentration of the library.

$$\text{Library concentration (nM)} = \frac{\text{Library concentration (ng/μL)} \times 10^6}{\text{Library length (bp)} \times 650}$$

   (2) Per the concentration measured, dilute the sample library to 4 nM with Nuclease-Free Water.
   (3) Proceed sample dilution and denaturation according to the matching Illumina sequencing kit (refer to the operation manual of each equipment).
   (4) The concentration of Phix Control V3 is more than 5% (for example: If the loading volume is 600 μL, the volume occupied by Phix Control V3 should be more than 30 μL).

(5) Library on-machine sequencing (according to the specification of instrument and matching reagent).

2. MGI sequencing platform

(1) The recommended amount of cyclic library input is 0.5 -1 pmol, and the required proportion of each library in the total library of 0.5 -1 pmol is calculated, and then the required amount of each library is calculated according to the following formula:

$$\text{Library input (ng)} = \frac{\text{Library length (bp)} \times 650 \times \text{proportion\%} \times \text{The recommended amount of cyclic library input (0.5 -1 pmol)}}{1000}$$

(2) According to the measured library concentration, the required input volume is calculated, and then mixed to obtain 0.5-1 pmol total library, the total volume is not more than 48 μL.

(3) Denaturation and cyclization of libraries according to the MGIEasy Universal Library Conversion Kit (App-A) (no need for split-conversion PCR, see the accompanying reagent manual for instructions).

(4) Proceed sample dilution and denaturation according to the matching sequencing kit (refer to the operation manual of each equipment).

(5) Library DNB preparation and on-machine sequencing (operation instructions refer to the accompanying reagent manual and instrument manual).

3. Sikun sequencing platform

(1) According to the library concentration measured by the Fluorometer, use the following formula to convert the molar concentration of the library.

$$\text{Library concentration (nM)} = \frac{\text{Library concentration (ng/μL)} \times 10^6}{\text{Library length (bp)} \times 650}$$

(2) Per the concentration measured, dilute the sample library to 4 nM with Nuclease-Free Water.

(3) Proceed sample dilution and denaturation according to the matching Sikun sequencing kit (refer to the operation manual of each equipment).

(4) Library on-machine sequencing (according to the specification of instrument and matching reagent).

Note: Store undiluted libraries at -15℃ to -25℃ for up to 7 days, the mixture of diluted libraries is suggested to be used right after it is ready.

VII. Bioinformatics Analysis

Transfer the Fastq files obtained by sequencing to the analysis server, perform data quality control, sequence alignment, annotation of gene mutation, CNV and MSI analysis-based on the Clinical NGS Data Analysis System of Xiamen Spacegen Co., Ltd.

Data Analysis

1. Standard of quality: For all sample DNA libraries, the target fragment should be in 200-350 bp, On Target should be ≥80%, Uniformity should be ≥75% and mean depth for somatic should be ≥5000×.
2. Mutated positive judgement criterion: In the result of somatic variations analysis, if effective depth is ≥500×. And mutation frequency is ≥1%, this mutation site is judged as positive mutation. Otherwise, it is judged as negative or below the detection limit.
3. The result of CNV: The Total Reads of the target genes accounted for the target region divided by the average of the percentage of the target gene with baseline samples, followed the ratio result is obtained. If the CNV-Ratio ≥2, it is judged as copy number amplifications. If the CNV-Ratio <2, it is judged as copy number amplifications negative or below the detection limit.
4. For MSI analysis, according to the MSI-Score to determine microsatellite state:
   (1) Microsatellite stable (MSS): MSI-Score <0.3.
   (2) Microsatellite instability (High-frequency MSI, MSI-H): MSI-Score ≥0.3.

Interpretation of Results

1. For the DNA negative control library, the target fragment should be in 200-350 bp, as well as On Target should be ≥80%, Uniformity should be ≥75%, moreover, mean depth ≥5000×. Otherwise, this test is invalidated.
2. For the DNA positive control library, the target fragment should be in 200-350 bp, as well as On Target should be ≥80%, Uniformity should be ≥75%, moreover, mean depth ≥5000×. Otherwise, this test is invalidated.
3. For the DNA sample libraries, the target fragment should be in 200-350 bp, each amplicon should have coverage, as well as On Target should be ≥80%, Uniformity should be ≥75%, moreover, mean depth for somatic should be ≥5000×. Otherwise, this mutation detection results are invalidated.
4. The grade of somatic variation based on the “Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in

Cancer: A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists" jointly formulated by AMP/ASCO/CAP in 2017 could divide into 4 types:

(1) Clear clinical significance: Diagnostic\prognostic marker of specific tumor or drugs recommended\approved in the professional guidelines.

(2) Potential clinical significance: Diagnostic\prognostic marker of specific tumor or drugs that has level A evidence of another tumor in the multiple small research.

(3) Unknown clinical significance: It is not found higher rates of variants in the general population and tumor databases, moreover, not has clear published evidence.

(4) Harmless or may be harmless clinical significance: It is found higher rates of variants in the general population and not published evidence.

Limitation of the Kit

1. For mutation sites that are not included in the kit, or the nucleic acids extracted from samples are stored longer than required, the results shall not be interpreted by the instruction.
2. The negative results cannot exclude the mutations. Too few tumor cells in the sample, excessive degradation, or the nucleic acids concentration in the library is below the detection limit can also cause a negative result.
3. Unreasonable sample collection, transportation, processing, improper operation and the experimental environment may lead to false negative or positive results.
4. Tumor tissue (cells) may have large heterogeneity, different test results may be obtained by sampling different parts.
5. The detection of gene copy number variation is easily affected by multiple factors such as tumor cell proportion, sequencing uniformity and chromosome ploidy.

Performance characteristics

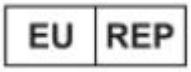
1. The kit should be neat in appearance, clearly labels, and no leakage.
2. When unfrozen, the reagents shall be clear, without sediments.
3. Negative reference conformity rate should be 100%.
4. Positive reference conformity rate should be 100%.
5. The kit allows the detection of 1% of specific gene mutations in 25 ng DNA tissue samples.
6. The detection limit of microsatellite instability is 5% tumor cell DNA content, and the national detection limit of 2 ng/μL microsatellite instability (MSI) can detect microsatellite instability of 5% tumor DNA content.

Warnings and Precautions

1. Please read the instruction carefully in prior to experiments.
2. Conduct experiments abided by laboratory regulations to reduce cross-contaminations of products or reagents. divide experiment areas into different function zones if possible.
3. Avoid repetitively freezing and thawing the reagents in the kit. Do not exceed a maximum of 5 freeze-thaw cycles.
4. The results of this kit will be affected by sample source, collection process, quality, transportation conditions, pre-treatment, etc., as well as the quality of the extracted nucleic, instrument types, operating environment, and the limitation of current molecular biotechnology. The factors and variables mentioned above would lead to false positive or false negative results. Users must be aware of the potential errors, accuracy and limitations that may exist during the process of detection.
5. The quality of nucleic acids is crucial, and the quality control of DNA should be performed after extraction, proceed to further steps immediately or store properly at -15℃ to -25℃.
6. Do not substitute any original reagents contained in the kit. Do not mix reagents with different Lots.
7. Pay special attention to the use of positive control and the use of filter pipette tips is highly recommended to avoid false-positive results caused by contamination of reagents.
8. Be cautious of contamination from external nucleic. Ensure to add the nucleic template before operating the positive control. Segregate areas for reagent preparation and sample processing. Use dedicated pipettes and pipette tips for reagent preparation and template addition, respectively.
9. Clean experiment areas before experiment with 10% hypochlorous acid followed by twice water rinsing. Sterilize the environment and pipettes with 10% hypochlorous acid, 75% ethanol, or UV radiation after experiment.

10. All reagents in use have potential hazard. It is recommended wearing proper protective suit and gloves. For first-use of this kit, you may receive training by our technical supports.
11. All samples including positive control in the kit should be considered as potential infectious substances which should be handled carefully.
12. Avoid using peripheral wells of PCR instrument. vacate holes or columns between samples to avoid cross-contamination.

Symbols

Symbol	Symbol definition
	Consult instructions for use.
	In vitro diagnostic medical device.
	Date of manufacture.
	Lot Number.
	Temperature limitation.
	Use by.
	This way up upright position of the packages for transport or storage.
	Keep dry.
	Keep away from sunlight.
	Manufacturer.
	European Authorised Representative.
	CE mark for European conformity.

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EU REP

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Appendix Table 1. Information of UDI Recognition Sequences

Strip Color	Number	i7 Sequence	i5 Sequence
Purple	UDI-1	TGCATAGC	TAGGATTC
	UDI-2	TCTATGCA	GTCGTTGC
	UDI-3	GTACGCAT	CCTCGCAT
	UDI-4	AGGTCCTG	AGAAGGCG
	UDI-5	CATGAGCT	ACGTCAGA
	UDI-6	AACTCTAG	CATCTGAT
	UDI-7	CCGGATGC	GTATCACG
	UDI-8	GTACGATA	TGCAACTA
Green	UDI-9	ATTCGATA	ATGGATCG
	UDI-10	CGTAGTAC	GCTGAATG
	UDI-11	GAGTACGT	CAACTGGC
	UDI-12	TCAGTGCG	TGCAGCAT
	UDI-13	CACACAGT	ACGACCAA
	UDI-14	GTGCATCG	CATTCGGC
	UDI-15	TGCGTCAC	GTATGATT
	UDI-16	ACATCGTA	TGCCTTCA
White	UDI-17	CGGAACGA	GCTGGCTT
	UDI-18	CCTGGCAC	ATAGAGAC
	UDI-19	ATATCGCT	CACATTGA
	UDI-20	GACAGTTG	TGGTCACG
	UDI-21	TGCCTATG	ACCTTCGG
	UDI-22	GTACCAGT	CGACCATC
	UDI-23	AATGTGCA	TAGCATCA
	UDI-24	TCGTATAC	GTTAGGAT
Yellow	UDI-25	CTGTGTGT	CGTCGTCT
	UDI-26	ACAGCACT	ATCCTAGC
	UDI-27	TATCAGTG	GAAGCCTG
	UDI-28	CGGTGTTA	TCGAAGTA
	UDI-29	GTCATCAC	ACCGGTAC
	UDI-30	GATGTCAG	CATTCAAT
	UDI-31	TCACAGCA	TGGTAGCA
	UDI-32	AGCACAGC	GTAATCGG

Appendix Table 2. Gene Information Included in the Kit

DNA Gene							
ARID1A	AKT1	CCNE1	CTNNB1	EPCAM	ESR1	FBXW7	HER2
KRAS	MECOM	MLH1	MSH2	MSH6	MYC	NRAS	PIK3CA
PIK3R1	PMS2	POLD1	POLE	PPP2R1A	PTEN	SMARCA4	SMARCB1
SOX17	SPOP	TERT	TP53				
MSI							
50 micro satellite loci							
CNV							
HER2		CCNE1		MECOM		MYC	TERT

Appendix Table 3. Positive control information of this kit

Gene Name	**Base Mutation**	**Amino Acid Mutation**	**Cosmic ID**	**Mutation Type**
ARID1A	c.827del	p.G276Efs*87	COSM133011	Deletion Mutation
POLE	c.1366G>C	p.A456P	COSM937318	Point Mutation
MSH6	c.3261del	p.F1088Sfs*2	COSM330655	Deletion Mutation
MLH1	c.1513del	p.S505Vfs*3	COSM26802	Deletion Mutation
PTEN	c.464A>G	p.Y155C	COSM5144	Deletion Mutation
PTEN	c.955_958del	p.T319*	COSM4898	Deletion Mutation
TP53	c.377A>G	p.Y126C	COSM11517	Point Mutation
TP53	c.267dup	p.S90Lfs*59	COSM131024	Duplication Mutation
Microsatellite Instability：MSI-H				