Medical equipment approval number: 30200BZI00007000

Machine equipment 17, Blood test equipment

Highly managed medical device General name: Somatic gene mutation analysis set (for determination of indication for antineoplastic drugs)

(JMDN code: 71059023)

ArcherMET companion diagnostic system

Reuse prohibition (analysis kit)

[Shape, structure and principle]
This product consists of the ArcherMET analysis kit and the Archer analysis software.

1. ArcherMET analysis kit

(1) ArcherMET ctDNA kit

Product Component
GMSK0012: Reagents for ArcherMET ctDNA
Complete end repair
Ligation Step 1
Ligation Step 2
First PCR
Second PCR Reactions, 1-8
Cleanup Beads
Cleanup Buffer
ArcherPure™ Purification Beads
GMSK0026: ArcherMET Sample Indexing Adapters 1-24
Sample Index Adapters 1-8
Sample Index Adapters 9-16
Sample Index Adapters 17-24
GMSK0027: ArcherMET Library Quantification
Library Quant Standard Set
Library Quant qPCR Mastermix
GMSK0013: Universal ctDNA Assay Gene Specific Primers
Universal ctDNA Assay GSP1
Universal ctDNA Assay GSP2

(2) ArcherMET RNA kit

Product Component
GMSK0014: Reagents for ArcherMET RNA
Random Priming
First Strand cDNA Synthesis
Second Strand cDNA Synthesis
Complete End Repair
Ligation Step 1
Ligation Step 2
First PCR
Cleanup Beads
Second PCR Reactions 1 - 8
RNA PreSeq® qPCR Mastermix
RNA PreSeq® Standard
Cleanup Buffer
ArcherPure™ Purification Beads
GMSK0026: ArcherMET Sample Indexing Adapters 1-24
Sample Index Adapters 1-8
Sample Index Adapters 9-16
Sample Index Adapters 17-24
GMSK0027: ArcherMET Library Quantification
Library Quant, Standard Set
Library Quant qPCR Mastermix
GMSK0015: Universal RNA Assay Gene Specific Primers
Universal RNA Assay, GSP1
Universal RNA Assay, GSP2

(3) Archer analysis software
  The Archer analysis software is provided to users online. Users are able to input sample information, instruct the corresponding analysis and receive results on the web application screen. The FASTQ file created during the sequencing is uploaded from the sequencer to the S3 bucket, and analyzed by the Bioinformatics pipeline as per the instructions received from the web application. The results are sent to the web application as a report.

<Principle>
This product consists of three main steps: (1) library preparation using proprietary Anchored Multiplex PCR (AMPTM) technology with extracted and quantified nucleic acid, (2) sequencing by synthesis using the Illumina MiSeqDx platform, and (3) sequencing data analysis by the Archer analysis software to produce the final report. Library preparation starts with an RNA or ctDNA input and ends with the quantification of the PCR library, moving on to the sequencer procedure. The Archer analysis software reads the sequencing output data (FASTQ file) and reports on the presence (or absence) of a skipping mutation in METex14.

[Use purpose or effect]
This product is intended to detect the presence (or absence) of the skipping mutation of the METex14 gene in RNA extracted from formalin-fixed paraffin-embedded (FFPE) cancer tissues or circulating DNA (ctDNA) extracted from plasma. The detection result of the METex14 mutation is used to determine Tepotinib adaptation capabilities.

[Usage method]
1. Usage method

1) Sample preparation

When using RNA as a sample

① Random priming: Priming of sample RNA is performed using a random hexamer. Incubation conditions are as follows:

Step	Temperature (°C)	Time (minutes)
1	65	5
2	4	Less than 15 minutes

② Synthesis of single-stranded cDNA: Synthesize single-stranded cDNA by reverse transcription using random-primed RNA as a template. Incubation conditions are as follows:

Step	Temperature (°C)	Time (minutes)
1	25	10
2	42	30
3	80	20
4	4	Maintenance

③ Synthesis of double-stranded cDNA: Synthesize double-stranded cDNA by nick translation, allowing RNAseH and DNA polymerase I to act on the double-stranded strands composed of the adjusted mRNA and cDNA. Incubation conditions are as follows:

Step	Temperature (°C)	Time (minutes)
1	16	60
2	75	20
3	4	Maintenance

When using ctDNA as a sample
ctDNA can be used in the analysis workflow without pre-processing.

2) Analysis workflow
3) After the above-mentioned pre-processing of the RNA sample is complete, the workflow procedure for RNA and ctDNA is the same.

④ End repair: Blunt the ends of the double-stranded DNA (dsDNA) fragments in the sample with T4 DNA polymerase, and then phosphorylate them with T4 polynucleotide kinase. Purify the reaction with ArcherPure. Incubation conditions are as follows:

Step	Temperature (°C)	Time (minutes)
1	37	30
2	4	Maintenance

⑤ 1st Ligation: Use the biotinylated dATP and the Klenow fragment to biotinylate and insert an A-tail in the 3' end of dsDNA. Purify once

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Please refer to the instruction manual at all times.

more with ArcherPure. Incubation conditions are as follows:

Step	Temperature (°C)	Time (minutes)
1	37	15
2	4	Maintenance

⑥ 2nd Ligation: Using T4 DNA ligase, add sample index adaptors with T overhang to the dsDNA inserted with an A-tail. Incubation conditions are as follows:

Step	Temperature (°C)	Time (minutes)
1	22	5
2	4	Maintenance

Isolate the AMP template using streptavidin-coated paramagnetic beads (Archer DX Cleanup beads). Incubation conditions are as follows:

Step	Temperature (°C)	Time (minutes)
1	75	10
2	4	Maintenance

⑦ 1st PCR (PCR1): In PCR1, amplify DNA fragments labeled with a sample index using a gene-specific primer (GSP1) and a universal primer (P5). After the PCR1 reaction is complete, purify with ArcherPure. Incubation conditions are as follows:

Step	Temperature (°C)	Time	Cycle
1	95	3 minutes	1
2	95	30 seconds	15
3	65	5 minutes	
4	72	3 minutes	1
5	4	Maintenance	1

⑧ 2nd PCR (PCR2): In PCR2, perform an amplification reaction using the product of PCR1 as a template and a gene-specific primer set (GSP2). Optimize the distance between gene-specific primers used for PCR1 and PCR2 using sensitivity and specificity as indices. In addition, in PCR2, add a P7 flow cell binding site and a second sample index tag, which are specific to Illumina products and are required when processing samples (libraries) using MiSeqDx. The product resulting from the PCR2 reaction purified by ArcherPure is hereinafter referred to as "library". Incubation conditions are as follows:

Step	Temperature (°C)	Time	Cycle
1	95	3 minutes	1
2	95	30 seconds	18
3	65	5 minutes	
4	72	3 minutes	1
5	4	Maintenance	1

⑨ Library quantification: Amplify the DNA strand and the appropriately diluted sample or library with a qPCR that targets the Illumina universal binding site added during library preparation. Use the best fit line of the DNA strand to convert average Cq value data into an absolute concentration. Perform a linear regression analysis of the sample is performed and calculate each library's concentrations. Adjust the concentrations of each library to a standard concentration and pool them. After repeating the amplification reaction on the finally obtained pool, use it in the sequencer at an appropriate concentration. Incubation conditions are as follows:

Step	Temperature (°C)	Time	Cycle
Initial denaturation	95	5 minutes	1
Denaturation	95	30 seconds	35
Annealing/ Extension/ Data collection	60	45 seconds	

⑩ Sequencing: Sequencing is performed using Illumina's MiSeqDx and Universal Sequencing reagents according to the procedure defined by Illumina.

⑪ Analysis and reporting: A series of pre-processing steps using the Archer analysis software must be performed on the sequence data file (FASTQ file) generated by MiSeqDx prior to analysis. After the file preparation is complete, a specific calling algorithm is run to detect point mutations, insertions or deletions, copy number variations, and RNA splice displacements. A clinical report describing the detected gene mutation is generated.

2. Input amount within a range that guarantees proper performance
ctDNA: At least 5 ng of purified ctDNA, ≤ 50 μL sample volume
RNA: 10-300 ng purified RNA, ≤ 20 μL sample volume

3. Medical devices used in combination
Use the following medical devices as a sequencer for conducting analysis with this product.
    Commercial name: MiSeqDx system
    General name: Genetic analyzer device
    Medical device manufacturing and commercial notification number: 13B1X10303000002
    Manufacturer/Vendor: Illumina Inc.

4. Analysis software requirements
The analysis software of this product is used with general-purpose IT devices that can use the Firefox or Google Chrome web browsers.

<Usage precautions>

(1) Precautions for handling plasma samples
1. Make sure to use a blood collection tube suitable for storing cell-free DNA and store the collected plasma at -80°C until use.

(2) Precautions for handling lyophilized reagents
1. After opening the pouch, carry out a visual inspection of the lyophilized pellet.
2. If the lyophilized pellet moves away from the tube's bottom, spin down for about 3-5 seconds to return the contents to the bottom of the tube.
3. Remove the required number of tubes and discard any unused tubes that remain after the pouch has been opened.
4. Do not use tubes containing more than one pellet.
5. Do not use tubes without any visible pellets.
6. Do not touch the lyophilized pellet with the pipette tip.
7. Add the sample/reagent to the lyophilized pellet while cooling on ice.
8. Vortex for about 2-5 seconds to dissolve and mix the lyophilized pellet. Excessive vortexing can adversely affect the first step of the assay.
9. Centrifuge for approximately 2-5 seconds to collect the contents at the bottom of the tube.
10. Keep the tubes on ice during the entire process.

(3) Precautions for use of Sample Index Adapters
1. To avoid cross-contamination between samples, refrain from simultaneously preparing a library containing the same Sample Index Adapter or a sequence pool of libraries containing the same Sample Index Adapter.
2. To prevent carry-over contamination between runs, refrain from using the same pair of Sample Index Adapters in successive sequencing runs.

(4) Precautions for use of ArcherPure™ Purification Beads
1. ArcherPure™ Purification Beads should be placed at room temperature (15-30°C) for 20-30 minutes before use, brought to room temperature and mixed to ensure homogenization.
2. When cleaning ArcherPure™ Purification Beads, make sure that all cleaning solvents have been removed prior to elution.

Please refer to the instruction manual at all times.

(5) Precautions on miscellaneous usage

1. • Use only components belonging to the same lot and do not replace any components.
2. • When freezing the sample, take note of the stop point in order to safely achieve the procedure (-30°C to -10°C).
3. • Reagent pouches are for single use only and should be discarded after use, including any unused remaining reactions.
4. • Observe the specified incubation times and temperatures that can affect library results.
5. • Manage the order in which samples are added and their locations. Assigning numbers and labels to the tubes is recommended.
6. • Prepare at least two libraries per batch. • Preparing at least 24 libraries per batch is recommended.

[Clinical Performance]

Based on the results of a pivotal phase II trial (VISION study) of Tepotinib in patients with advanced NSCLC with a METex14 skipping mutation, clinical trials were conducted to determine the match rate of METex14 skipping mutations between the control test (CTA) and the product. In addition, the clinical usefulness of the product was evaluated by examining the confirmed overall response (OR) or partial response (PR) data as determined by the investigator in patients with positive METex14 skipping mutation.

Table 1: Match rate between this product and the control test for plasma samples

Match rate criteria	Match rate (n/N)	95% CI*
OPA	91.48 (161/176)	86.33, 95.15
PPA	78.69 (48/61)	66.32, 88.14
NPA	98.26 (113/115)	93.86, 99.79

* 95% confidence interval accuracy using the Clopper-Pearson method

Table 2: Match rate between this product and the control test for tissue samples

Match rate criteria	Match rate (n/N)	95% CI*
OPA	97.04 (164/169)	93. 23, 99. 03
PPA	93.88 (46/49)	83. 13, 98. 72
NPA	98.33 (118/120)	94. 11, 99. 80

* 95% confidence interval accuracy using the Clopper-Pearson method

Table 3: Clinical utility

OR rate (CR/PR)	CTA+ n/N % (95%CI)	(TBx and/or LBx)	
		CTA+ /CDx+ n/N % (95% CI)	CTA+ /CDx- n/N % (95% CI)
LBx	30/66 45.5 (33.1, 58.2)	20/48 41.7 (27.6, 56.8)	13/28 46.4 (27.5, 66.1)
TBx	26/60 43.3 (30.6,56.8)	20/47 42.6 (28.3, 57.8)	1/4 25.0 (0.6, 80.6)
LBx and TBx	42/99 42.4 (32.5, 52.8)	34/78 43.6 (32.4, 55.3)	5/11 45.5 (16.7, 76.6)

CTA +: Positive in control test, CDx +/-: Positive/negative in product
* 95% confidence interval accuracy using the Clopper-Pearson method

Table 4: Subset of VISION trial patients with both LBx and TBx samples.

	TBx+	TBx-
LBx+	18	1
LBx-	19	58

*Sample agreement was analyzed retrospectively. VISION enrolled patients based on independent testing by either LBx- or TBx- assays.

In the VISION trial, there were 96 patients with both LBx and TBx samples available for testing with the product. From these patient samples, overall agreement between LBx and TBx was 78.95%. The prevalence of METex14 skip positivity in NSCLC is approximately 3%. Accounting for this prevalence, the negative predictive value from the LBx test is 98.4%. Patients who tested positive with either LBx or TBx demonstrated improved clinical outcomes (Table 3).

[Storage and shelf life]

<Storage method>

Storage temperature: 2-8℃

<Shelf life>

3 months

[Names of manufacturer and vendor]

Appointed manufacturer and vendor: Corbridge Co., Ltd.

Foreign manufacturer: ArcherDX, Inc. (United States)