AmoyDx® HANDLE Melanoma NGS Panel

Instructions for Use

For Research Use Only. Not for use in diagnostic procedures.

REF 8.06.0156 24 tests/kit For Illumina NextSeq 500, NextSeq 550, MiSeq, MiSeqDx (RUO Mode), iSeq 100

Amoy Diagnostics Co., Ltd.
No. 39, Dingshan Road, Haicang District,
361027 Xiamen, P. R. China
Tel: +86 592 6806835
Fax: +86 592 6806839
E-mail: sales@amoydx.com
Website:www.amoydx.com

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Background

Melanoma is a deadly form of cutaneous cancer primarily driven by genetic mutations. The BRAF gene is found frequently mutated which implicated in different mechanisms underlying melanomagenesis. The V600E mutation is taking over 90 % of BRAF mutation in melanoma and leads to uncontrolled cell growth[1]. Mutations in the CTNNB1 gene are less common but can activate the Wnt signaling pathway, aberrant activation of the Wnt signaling pathway has been reported in different human tumor types, including malignant melanomas[2]. GNA11 and GNAQ mutations are implicated in uveal melanoma, a subtype of melanoma that occurs in the eye. They activate the MAPK pathway, driving tumor development leading to increased risk of death in uveal melanoma[3]. HRAS, KRAS, and NRAS mutations in melanoma activate the MAPK pathway, promoting uncontrolled cell growth and cancer progression. These mutations are associated with more aggressive forms of the disease and can influence treatment decisions, such as targeted therapies or immunotherapies. However, KRAS and HRAS are found mutated in 2% and 1% of cases respectively, NRAS mutations (such as Q61R, Q61K) are found in 15%~20% of melanomas, preclinical data from current therapies for NRAS-mutant melanoma shows still limited benefit in overall survival[4-5]. Mutations in the KIT gene are often detected in mucosal melanoma and acral lentiginous melanoma subtypes. The most common KIT mutation in melanoma is the L576P mutation. Detection of KIT mutations is crucial as they can influence treatment decisions, potentially leading to targeted therapies with drugs like imatinib in certain cases[6]. The TERT gene encodes the telomerase enzyme, which is often upregulated in melanoma, allowing for limitless cell division and promoting tumor growth, major in tumors like melanoma, glioblastoma, and hepatocellular carcinoma, with melanoma having the most prevalent noncoding regulatory region mutations[7]. NGS in melanoma detection provides specific genetic alteration information that is vital for personalized treatment strategies[8].

Intended Use

The AmoyDx® HANDLE Melanoma NGS Panel is a next-generation sequencing (NGS) based assay intended for qualitative detection of single nucleotide variants (SNVs), insertions and deletions (InDels) in key melanoma tumour genes (see Appendix), using DNA isolated from formalin-fixed paraffin embedded (FFPE) tumour tissue specimens.

The kit is intended to be used by trained professionals in a laboratory environment.

Principles of the Procedure

The test kit is based on Halo-shape ANnealing and Defer-Ligation Enrichment system (HANDLE system) 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.

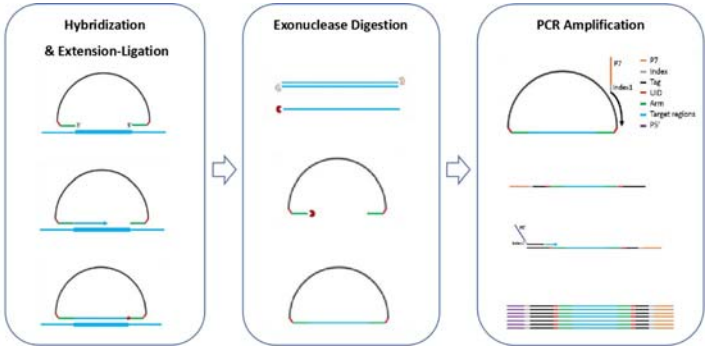

Figure 1. Principle of library construction (HANDLE system)

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

No.	Abbreviation	Content	Main Ingredient	Quantity
1	1-Hyb	**MEL-Probe**	Oligonucleotides	28 µL/tube ×1
2	2-Hyb	**MEL-Hybridization Buffer**	Tris-HCl, K^+, Mg^{2+}	28 µL/tube ×1
3	3-EL	**MEL-Extension Ligation Master Mix**	DNA polymerase, dNTPs, DNA Ligase, Ligation buffer	40 µL/tube ×1
4	4-ED	**MEL-Exonuclease A**	DNA Exonuclease	40 µL/tube ×1
5	5-ED	**MEL-Exonuclease B**	DNA Exonuclease	28 µL/tube ×1
6	6-Amp	**MEL-PCR Master Mix**	dNTPs, DNA polymerase, PCR buffer	600 µL/tube ×1
7	502-511	**MEL-S5 Primer** *	Oligonucleotides	5 µL/tube×8
8	716-729	**MEL-N7 Primer** *	Oligonucleotides	5 µL/tube×12
9	PC	**MEL-Positive Control** **	DNA	60 µL/tube×1
10	NC	**MEL-Negative Control** ***	DNA	60 µL/tube×1

** For labeling and sequence information of the MEL-S5 and MEL-N7 primers, refer to Appendix Table S3.*

*** The positive variants in the MEL-Positive Control are listed in Appendix Table S4.*

**** The MEL-Negative Control shows a negative result in the detection range of this kit.*

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°C±5°C.

The shelf-life of the kit is twelve months. Repeated freezing and thawing should be avoided, and the maximum freeze-thaw cycle is five.

Additional Reagents and Equipment Required but Not Supplied

1) PCR instrument: Applied Biosystems™ 2720 Thermal Cycler, MiniAmp™ Thermal Cycler or Hangzhou Bioer TC-E-48D Gene Pro Thermal Cycler is recommended.
2) DNA quantification kit: QuantiFluor dsDNA System (Promega), Qubit® dsDNA HS Assay Kit (Thermo Fisher Scientific) or iQuant™ ssDNA Quantitation Kit (GeneCopoeia) is recommended.
3) Fluorometer: Quantus™ Fluorometer (Promega), or Qubit 2.0/3/4 Fluorometer (Thermo Fisher Scientific) is recommended.
4) DNA extraction kit: AmoyDx® Magnetic FFPE DNA Extraction Kit is recommended for DNA extraction from FFPE sample. It is recommended to use RNase A to degrade RNA during the DNA extraction.
5) DNA purification kit: Agencourt AMPure XP Kit (Beckman Coulter) or CleanNGS magnetic beads (Vdobiotech) is recommended.
6) Sequencing reagent: Illumina 300 cycles (Paired-End Reads, 2×150 cycles) related reagents is recommended.
7) Sequencer: Illumina MiSeq, MiSeqDx (RUO Mode), NextSeq 500, NextSeq550, or iSeq 100 is recommended.
8) Illumina PhiX Control V3 or TG PhiX Control Kit v3.
9) Capillary electrophoresis analyzer and related kit: Agilent 2100 Bioanalyzer system and Agilent DNA 1000 Reagents (Agilent Technologies) or Agilent High Sensitivity DNA Kit (Agilent Technologies); or Agilent 2200 TapeStation and D1000 ScreenTape/Reagents (Agilent Technologies) or High Sensitivity D1000 ScreenTape/Reagents (Agilent Technologies); or LabChip GX Touch and DNA High Sensitivity Reagent Kit (PerkinElmer); or E-GelTM Power Snap Electrophoresis System (Thermo Fisher Scientific) and E-GelTM EX Agarose Gels, 2% (Thermo Fisher Scientific) is recommended.
10) Magnetic Stand: DynaMag™-2 Magnet (Thermo Fisher Scientific) or DynaMag™-96 Side Magnet (Thermo Fisher Scientific) is recommended.
11) Vortex mixer.
12) Mini centrifuge.
13) Nuclease-free centrifuge tubes.
14) Nuclease-free PCR tubes.
15) Nuclease-free filtered pipette tips.
16) Nuclease-free water.

17) RNase A (DNase and Protease free).

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

19) Absolute ethanol (Analytical Reagent).

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

Precautions and Handling Requirements

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.
- Please check the compatible PCR instruments prior to use.
- DO NOT use the kit or any kit component after the expiry date.
- DO NOT use any other reagents from different lots.
- DO NOT use any other reagents from 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.

Decontamination and Disposal

- The kit contains positive control; strictly distinguish the positive control from other reagents to avoid contamination which may cause false positive.
- 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 reagents, and waste must be disposed properly.

Cleaning

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

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Specimen Preparation

- Sample DNA should be extracted from melanoma FFPE tissue specimens.
- 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 2 years.
- It is recommended that the tumor cell content is no less than 20%.
- For surgical tumor resection specimens, it is recommended to use 2~6 unstained slides (5~10 μM thick) for DNA extraction. For tissue biopsy specimens, it is recommended to use 10~15 unstained slides (5 μM thick) for DNA extraction. The number of FFPE slides used for DNA extraction can be appropriately adjusted based on sample quality and tumor size.
- It is recommended to use a commercialized extraction kit to perform the DNA extraction, and use RNase A to degrade RNA during the DNA extraction. After extraction, measure the concentration of extracted DNA using Quantus™ or Qubit®. The DNA concentration should be more than 3.75 ng/μL, and the 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 library preparation immediately, if not, the DNA 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 MEL-Positive Control (PC) and a MEL-Negative Control (NC) in the process of library construction, sequencing, and 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 can be used for the quality control of the library construction process, while for sequencingor data analysis process is not needed.*
- *During the following DNA library preparation process, please use the corresponding adaptor in the PCR instrument to avoid PCR product evaporation.*
- *It is recommended to use fluorescent dye method (Qubit or Quantus Fluorometer) for all the DNA concentration measurement step.*

1. DNA pre-denaturation

1.1 Take out the DNA samples, MEL-Positive Control, MEL-Negative Control and TE-low solution, thaw the reagents at room temperature. When the reagents are completely thawed, mix well by vortexing for 10-15 sec and centrifuge briefly, then keep the tube on ice. Prepare the pre-denaturation solution in a 0.2 mL PCR tube according to Table 2.

Table 2. Pre-denaturation reaction

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

Note:

- *"χ" stands for the volume of 30~100 ng DNA (50 ng is recommended).*
- *For Positive Control and Negative Control, χ=8.*
- *For NTC, use the TE-low solution of 8 μL, χ=0.*

1.2 Mix the solution thoroughly by vortexing for 10-15 sec or pipetting, then spin down briefly, place the samples in a thermocycler, set the reaction volume as 8 μL and perform the following program: 98°C for 5 min, then put the tubes on ice immediately (≥1 min).

2. Hybridization

2.1 Take out the (1-Hyb) **MEL-Probe** and (2-Hyb) **MEL-Hybridization Buffer** and thaw the reagents at room temperature. When the reagents are completely thawed, mix well by vortexing for 10-15 sec and centrifuge briefly, then keep the tube on ice.

2.2 Prepare the hybridization reaction mix on ice by adding the following components according to Table 3.

Table 3. Hybridization reaction

Reagent	Volume
(1-Hyb) MEL-Probe	1 μL
(2-Hyb) MEL-Hybridization Buffer	1 μL
Pre-denaturation product (from step 1.2)	8 μL
Total	**10 μL**

Note:

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

2.3 Mix the solution thoroughly by vortexing for 10-15 sec or pipetting, then spin down briefly, place the samples in a thermocycler, set the reaction volume as 10 μL and perform the following program: 95°C for 5 min, 60°C for 2 h, 4°C hold.

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

3. Extension-Ligation

3.1 Take out the (3-EL) **MEL-Extension Ligation Master Mix** and thaw the reagent on ice. When the reagents are completely thawed, mix well by vortexing for 10-15 sec 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 1.5 μL (3-EL) **MEL-Extension Ligation Master Mix** into the PCR tubes, mix the solution thoroughly by vortexing for 10-15 sec or pipetting, and spin down briefly, then place the sample in a thermocycler (recommend to preheat the thermocycler to 50°C), set the reaction volume as 12 μL and

perform the following program: 60°C for 10 min, 4°C hold. Then proceed directly to the exonuclease digestion.

Note:

- *Perform the subsequent exonuclease digestion step immediately when the extension-ligation step is finished.*
- *It is recommended to immediately transfer the tube on ice box after the extension-ligation program is finished, as high temperature like room temperature may increase the non-specificity.*

4. Exonuclease Digestion

4.1 Take out the (4-ED) **MEL-Exonuclease A** and (5-ED) **MEL-Exonuclease B**, mix well by vortexing for 10-15 sec and centrifuge briefly, then keep the tube on ice.

4.2 Take out the extension-ligation product from step 3.2, prepare the exonuclease digestion reaction mix on ice by adding the following components according to Table 4.

Table 4. Exonuclease digestion reaction

Reagent	Volume
(4-ED) MEL-Exonuclease A	1.5 μL
(5-ED) MEL-Exonuclease B	1 μL
Extension-Ligation product (from step 3.2)	11.5 μL
Total	**14 μL**

4.3 Mix the solution thoroughly by vortexing for 10-15 sec or pipetting, and spin down 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:

- *It is recommended to prepare **freshly ready-to-use premix** of MEL-Exonuclease A and MEL-Exonuclease B for precise pipetting when perform three or more samples simultaneously.*
- *The products of exonuclease digestion should be stored at 2~8°C for no more than 20 hours if not proceed to the next step.*

5. PCR Amplification

5.1 Take out the **MEL-S5 Primer, MEL-N7 Primer** and (6-Amp) **MEL-PCR Master Mix** and thaw the reagents at room temperature. When the reagents are completely thawed, mix well by vortexing for 10-15 sec and centrifuge briefly, then keep the tube on ice.

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

Table 5. PCR amplification reaction

Reagent	Volume
(6-Amp) MEL-PCR Master Mix	25 μL
MEL-S5 Primer	1.5 μL
MEL-N7 Primer	1.5 μL
Nuclease-free water	8 μL
Exonuclease digestion product (from step 4.3)	14 μL
Total	**50 μL**

Note:

- *Each of the MEL-S5 Primer or MEL-N7 Primer has a different index sequence. Use different combination of MEL-S5 Primer and MEL-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.*
- *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 for 10-15 sec or pipetting, and spin down 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°C	1 min	1
98°C	20 s	25
61°C	30 s	
72°C	20 s	
72°C	5 min	1
4°C	∞	1

Note: *The PCR products should be stored at 2~8°C for no more than 20 hours if not proceed to the next step.*

6. Purification

6.1 Take out the AMPure XP beads (or Clean NGS magnetic beads), and equilibrate them to room temperature for 30 min, and vortex the bottle of the beads for 10~15 sec to resuspend any magnetic particles that may have settled.

6.2 Add **37 μL resuspended beads** and **40 μL PCR products** into 1.5 mL centrifuge tubes, mix thoroughly by vortexing for 10-15 sec or pipetting, then incubate the mixture at room temperature for 5 min.

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 while in the magnetic rack. 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 rack. 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 20~30 μL TE-low solution, mix thoroughly by vortexing for 10-15 sec or pipetting, and incubate for 3 min at room temperature.

6.8 Put the tube in the magnetic rack 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: *It is recommended to store the library at -20°C if not proceed directly to sequencing.*

7. Library Quality Control (QC)

7.1 Library concentration QC: Quantify the library concentration by Quantus™ or Qubit® Fluorometer, the DNA total amount should be no less than 60 ng. (e.g, elute DNA target with 30 μL TE-low solution, the DNA concentration should be no less than 2 ng/μL)

Note: *For NTC, the DNA total amount should be less than 60 ng.*

7.2 Library fragment QC (optional): Assess the library quality on Agilent 2100 Bioanalyzer (or equivalent), the main peak size of the DNA fragment should be at 200~400 bp, as shown in Figure 2.

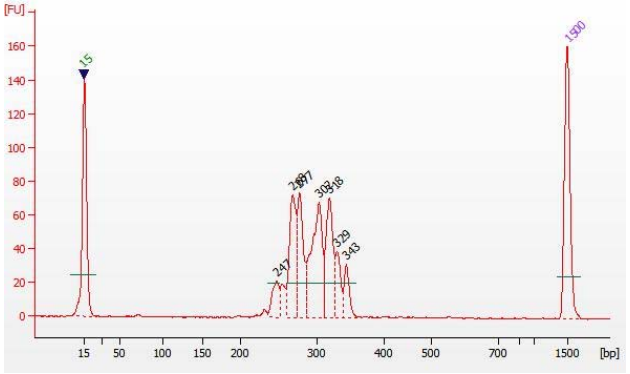

Figure 2. Example of library size distribution on a Bioanalyzer

Note:

- *The library distribution shown in the Figure 2 above was assessed using Agilent 2100 Bioanalyzer system and Agilent DNA 1000 Reagents. The peak at 15 bp stands for the lower marker, and the peak at 1500 bp stands for the upper marker.*
- *The no template control (NTC) library should have a DNA amount below 60 ng, and no targeted DNA fragment size (200~400 bp). If not, there may be contamination during the experiment process, the test is unqualified and the test should be repeated.*
- *If the library QC pass, then move to sequencing. If not, the library should be reconstructed. Please first check whether it is caused by operating errors or the DNA quality is poor. If no operating errors, that may be caused by poor DNA quality, in this case, it is recommended to extend the hybridization time (60°C for 12~18 h) to rebuild the library, or re-collect and re-test the sample. For failed samples, it's recommended to try dividing the DNA sample into separate tubes to rebuild the library (such as 33.3 ng×3 tubes), and in the library purification step, the products derived from the same sample are combined and purified.*

8. Sequencing

Illumina 300 cycles (Paired-End Reads, 2×150 cycles) reagent is recommended for sequencing. The recommended percentage of Illumina PhiX Control v3 should be more than 1% and no more than 50% maximum. The sequencing data per sample should be no less than 25 Mb. The suggested sample quantity per run is listed in Table 7.

Table 7. Recommended sequencer and sample quantity per run

Sequencer	Flow Cell	Read Length	Sample Quantity/Run
iSeq 100	i1	2× 150 bp	~48
MiSeq/MiSeqDx (RUO Mode)	Micro	2× 150 bp	~48
MiSeq/MiSeqDx (RUO Mode)	V2/V3	2× 150 bp	up to 96#
NextSeq 500/NextSeq 550	Mid/High	2× 150 bp	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
iSeq 100	30~40 pM
MiSeq/MiSeqDx (RUO Mode)	5~8 pM
NextSeq 500/NextSeq 550	0.6~1.0 pM

Note: *The concentration converting formula:*

$$Library\ Concentration\ [nM] = \frac{Library\ Concentration\ [ng/\mu L] \times 10^6}{660 \times 290}$$

9. Data Analysis

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

Select the appropriate analysis module ADXHS-tMEL or ADXHS-tMEL7.

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 the quality of the sequencing batch is unqualified, it is recommended to use the remaining library of the sample batch to re-sequence or re-construct the library for sequencing. The Q30 bases in different sequencers may differ, but should not be less than 75%.

Data Quality Control (QC):

The qualified criteria for data QC is shown below.

1) The "CleanQ30" should be ≥ 75%. If not, the sample is failed, and it is recommended to use the remaining library of the sample to re-sequence or re-construct the library for sequencing.
2) "Depth" of each sample from summary sheet should be no less than 300×.

Result Interpretation

- The Cut-off metrics are listed in Table 9.

Table 9. Cut-Off Metrics

Analysis Module*	Depth	AltDepth	Freq
ADXHS-tMEL	≥ 30×	≥ 8	≥ 3%
ADXHS-tMEL7	≥ 30×	≥ 8	≥ 3%

Analysis Module: see Appendix.

Note*:*

- *Q30: one base call in 1,000 is predicted to be incorrect meaning a base call accuracy of 99.9%.*
- *Depth (shown in SNVIndel sheet): The effective depth of the variant site after UMI calibration.*
- *AltDepth: Depth of mutant allele after UMI calibration.*
- *Freq: Frequency of mutant allele.*

Performances

Limit of Detection (LoD) for SNV/InDel detection is 5% allele frequency.

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 FFPE tissue samples.
3) Reliable results are dependent on proper sample processing, transport, and storage.
4) Negative results can not completely exclude the existence of mutated genes. Low tumor cell content, severe DNA degradation or the frequency under the limit of detection may also cause inaccurate result.
5) Sampling of different sites of tumor tissue may lead to different test results due to tumor heterogeneity.
6) False negative results may occur when InDels occurs on the probe binding regions.
7) The longest InDels mutation covered in the sample used for validation is 57 bp. InDels longer than 57 bp has not been fully validated, the detection ability may decrease as the length of the InDels increases.
8) Please strictly follow the sequence data volume recommended in the IFU, otherwise, it may lead to inaccurate result.

Reference

1. Ascierto PA, Kirkwood JM, Grob JJ, et al. The role of BRAF V600 mutation in melanoma. J Transl Med. 2012;10:85.
2. Reifenberger J, Knobbe CB, Wolter M, et al. Molecular genetic analysis of malignant melanomas for aberrations of the WNT signaling pathway genes CTNNB1, APC, ICAT and BTRC. Int J Cancer. 2002;100(5):549-556.
3. Piaggio F, Croce M, Reggiani F, et al. In uveal melanoma Gα-protein GNA11 mutations convey a shorter disease-specific survival and are more strongly associated with loss of BAP1 and chromosomal alterations than Gα-protein GNAQ mutations. Eur J Cancer. 2022;170:27-41.
4. Milagre C, Dhomen N, Geyer FC, et al. A mouse model of melanoma driven by oncogenic KRAS. Cancer Res.

2010;70(13):5549-5557.

5. Muñoz-Couselo E, Adelantado EZ, Ortiz C, García JS, Perez-Garcia J. NRAS-mutant melanoma: current challenges and future prospect. Onco Targets Ther. 2017;10:3941-3947.
6. Pham DDM, Guhan S, Tsao H. KIT and Melanoma: Biological Insights and Clinical Implications. Yonsei Med J. 2020;61(7):562-571.
7. Guo Y, Chen Y, Zhang L, et al. TERT Promoter Mutations and Telomerase in Melanoma. J Oncol. 2022;2022:6300329. Published 2022 Jul 19.
8. NCCN Guidelines Version 2.2023 Melanoma: Cutaneous

Symbols

	Manufacturer		Catalogue Number
	Batch Code		Use By
	Contains Sufficient for <n> Tests		Temperature Limitation
	Consult Instructions For Use		Keep Dry
	This Way Up		Fragile, Handle With Care

Appendix

Table S1. Target regions of ADXHS-tMEL

No.	Gene	Transcripts	Mutation	Related Exons
01	*BRAF*	NM_004333	SNV, InDel	Exon 11,15
02	*CTNNB1*	NM_001904	SNV, InDel	Exon 3
03	*GNA11*	NM_002067	SNV, InDel	Exon 4,5
04	*GNAQ*	NM_002072	SNV, InDel	Exon 4,5
05	*HRAS*	NM_001130442	SNV, InDel	Exon 2,3
06	*KIT*	NM_000222	SNV, InDel	Exon 9,11,13,17,18
07	*KRAS*	NM_033360	SNV, InDel	Exon 2,3,4
08	*NRAS*	NM_002524	SNV, InDel	Exon 2,3,4
09	*TERT*	NM_198253	SNV	c.-124C > T (C228T),c.- 146C > T (C250T)

Table S2. Target regions of ADXHS-tMEL7

No.	Gene	Transcripts	Mutation	Related Exons
01	*BRAF*	NM_004333	SNV, InDel	Exon 11,15
02	*GNA11*	NM_002067	SNV, InDel	Exon 5
03	*GNAQ*	NM_002072	SNV, InDel	Exon 4,5
04	*HRAS*	NM_001130442	SNV, InDel	Exon 2,3
05	*KIT*	NM_000222	SNV, InDel	Exon 9,11,13,17,18
06	*NRAS*	NM_002524	SNV, InDel	Exon 2,3,4
07	*TERT*	NM_198253	SNV	c.-124C > T (C228T),c.- 146C > T (C250T)

Table S3. Index sequence information for primers

Name	Primer Index Name	Primer Index Information	Illumina Nextera XT v2 Set B No.
716	MEL-N716	TAGCGAGT	N716
718	MEL-N718	GTAGCTCC	N718
719	MEL-N719	TACTACGC	N719
720	MEL-N720	AGGCTCCG	N720
721	MEL-N721	GCAGCGTA	N721
722	MEL-N722	CTGCGCAT	N722
723	MEL-N723	GAGCGCTA	N723
724	MEL-N724	CGCTCAGT	N724
726	MEL-N726	GTCTTAGG	N726
727	MEL-N727	ACTGATCG	N727
728	MEL-N728	TAGCTGCA	N728
729	MEL-N729	GACGTCGA	N729

Name	Primer Index Name	Primer Index Information	Illumina Nextera XT v2 Set B No..
502	MEL-S502	CTCTCTAT	S502
503	MEL-S503	TATCCTCT	S503
505	MEL-S505	GTAAGGAG	S505
506	MEL-S506	ACTGCATA	S506
507	MEL-S507	AAGGAGTA	S507
508	MEL-S508	CTAAGCCT	S508
510	MEL-S510	CGTCTAAT	S510
511	MEL-S511	TCTCTCCG	S511

Table S4. HotSpot mutations in MEL-Positive Control

No.	Gene	Mutation	Analysis Module
01	*BRAF*	Exon15:c.1799T>A:p.(V600E)	ADXHS-tMEL
02	*NRAS*	Exon3:c.181C>A:p.(Q61K)	
03	*CTNNB1*	Exon3:c.133_135del:p.(S45del)	
04	*KRAS*	Exon2:c.38G>A:p.(G13D)	

No.	Gene	Mutation	Analysis Module
01	*BRAF*	Exon15:c.1799T>A:p.(V600E)	ADXHS-tMEL7
02	*NRAS*	Exon3:c.181C>A:p.(Q61K)	