AmoyDx® *MET* Mutation Detection Kit

Instructions for Use

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

REF 8.01.0123 24 tests/kit For Stratagene Mx3000P™, ABI7500, LightCycler480 II, SLAN-96S, Rotor-Gene Q (72 wells)

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

Version: B1.1
May 2023

Background

Lung cancer is one of the most common malignant tumor, and 80~85% of lung cancers are non-small cell lung cancer (NSCLC) [1]. There are many driver mutations in NSCLC, and *MET* alterations that result in exon 14 skipping are also found. *MET* exon 14 skipping mutations have been identified in approximately 3~4% of lung adenocarcinoma patients [2-5]. Studies have shown that NSCLC patients harboring *MET* mutations causing exon 14 skipping response to *MET*-targeted therapy, as the presence of exon 14 skipping mutation in the *MET* gene is associated with increased sensitivity of *MET* inhibitors [5-9].

Intended Use

The AmoyDx® *MET* Mutation Detection Kit is a real-time PCR assay for qualitative detection of exon 14 skipping mutation in *MET* gene in human total RNA extracted from NSCLC formalin-fixed paraffin-embedded (FFPE) tissue samples (see Table 1).
The kit is for research use only, and intended to be used by trained professionals in a laboratory environment.

Table 1 Details of *MET* exon 14 skipping mutation

Spliced Gene & Exon	Spliced Gene & Exon
MET exon13	*MET* exon15

Principles of the Procedure

The kit comprises specific primers and fluorescent probes to detect gene mutation in real-time PCR assay, which combines reverse transcription and PCR amplification in one step. The target region of FFPE RNA is transcribed into cDNA with the action of reverse transcriptase and specific primers. The *MET* variant cDNA is amplified by specific primers and the mutant amplicon is detected by fluorescent probe labeled with FAM, while reference gene amplicon is detected by fluorescent probe labeled with VIC.
This kit is composed of Reaction Mix, sufficient Enzyme Mix and Positive Control.

1) The contents in **MET Reaction Mix A** and **MET Reaction Mix B** formed a mutation detection system and an internal control system. The mutation detection system is used to detect the exon 14 skipping mutation of *MET* gene. The internal control system is used to detect *MET* gene to assess *MET* gene expression level and monitor the accuracy of experimental operation.
2) The **MET Enzyme Mix** contains Reverse Transcriptase for cDNA synthesis, Taq DNA polymerase for PCR amplification and uracil-N-glycosylase which works at room temperature to prevent PCR amplicon carryover contamination.
3) The **MET Positive Control (PC)** contains recombinant *MET* gene with exon 14 skipping mutation and wild-type DNA.

Kit Contents

This kit contains the following materials (Table 2).

Table 2 Kit Contents

Content	Main Ingredients	Quantity
MET Reaction Mix A	Primers, Probes, Mg^{2+}, dNTPs	800 µL/tube ×1
MET Reaction Mix B	PCR Buffer	160 µL/tube ×1
MET Enzyme Mix	Reverse Transcriptase Taq DNA Polymerase, Uracil-N-Glycosylase	21 µL/tube ×1
MET Positive Control	Plasmid DNA	100 µL/tube ×1

Storage and Stability

The kit requires shipment on frozen ice packs. All components of the kit should be stored immediately upon receipt at -20±5℃ and protected from light.
The shelf-life of the kit is twelve months. The maximal number of freeze-thaw cycle is five.

Materials Required But Not Supplied

1) Compatible PCR instrument: Stratagene Mx3000P™, ABI7500, LightCycler480 II, SLAN-96S, or Rotor-Gene Q (72 wells).

2) RNA extraction kit. We recommend to use AmoyDx® FFPE RNA Kit for FFPE tissue specimens.
3) Spectrophotometer for measuring RNA concentration.
4) Mini centrifuge with rotor for centrifuge tubes.
5) Mini centrifuge with rotor for PCR tubes.
6) Vortexer.
7) Nuclease-free centrifuge tubes.
8) Nuclease-free PCR tubs and caps.
9) Adjustable pipettors and filtered pipette tips for handling RNA.
10) Tube racks.
11) Disposable powder-free gloves.
12) Sterile, nuclease-free water.

Precautions and Handling Requirements

Precautions

- Please read the instruction carefully and become familiar with all components of the kit prior to use. Strictly follow the instruction during operation.
- Please check the compatible real-time PCR instruments prior to use.
- 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 reagent in the other test kits.

Safety Information

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

Decontamination and Disposal

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

Cleaning

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

Instrument Setup

- Setup the reaction volume as 40 μL.
- For Stratagene Mx3000P™, please select “Collect fluorescence data” as FAM and HEX, set up the Fliter Set Gain Settings of FAM and HEX-JOE as 2.

- For LightCycler480 II, if there is fluorescence crossover on instrument, fluorescence calibration is also required. To run the assays on a LightCycler machine, please use the Roche 480 adaptor, available from BIOplastics, Cat No. B79480.
- For ABI7500 instrument please set up as follows: Reporter Dye: FAM, VIC; Quencher Dye: TAMRA; Passive Reference: NONE.
- For SLAN-96S instrument, please set up as follows: Fluorophores/Dyes: FAM, VIC. During the result interpretation, select “Selected Wells” for “Y-Axis Scaling Auto-adjust By” and “Absolute fluorescence Method” for “Normalization algorithm”.
- Refer to the operations manual of the real-time PCR instrument for detailed instructions.
- We recommend that for all PCR instruments in use, a fluorescence calibration should be conducted once a year.

Assay Procedure

1. RNA Extraction

The specimen material must be extracted RNA from NSCLC FFPE tissue samples. RNA extraction kit is not included in the kit. Before RNA extraction, it is essential to use a standard pathology methodology to ensure tumor sample quality and make sure that there are tumor cells in the FFPE tissue samples. Carry out the RNA extraction according to the instructions of the RNA extraction kit.

The OD value of extracted RNA should be measured using the spectrophotometer after extraction (NanoDrop 1000/2000 spectrophotometer is recommended).

The OD_{260}/OD_{280} value should be between 1.8~2.1 and total RNA concentration should be between 20~500 ng/μL.

Note:

- *The FFPE tissue should be handled and stored properly. The storage time should preferably be less than 2 years.*
- *The extracted RNA should be used immediately. If not, it should be stored at -20±5 ℃.*

2. Mutation Detection

1) Take **MET Reaction Mix A**, **MET Reaction Mix B**, **MET Enzyme Mix** and **MET Positive Control (PC)** as need out of the kit from the freezer.
2) Thaw **MET Reaction Mix A**, **MET Reaction Mix B** and **MET PC** at room temperature. When the reagents are completely thawed, mix each reagent thoroughly by vortexing and centrifuge for 5~10 seconds to collect all liquid at the bottom of the tube.
3) Centrifuge **MET Enzyme Mix** for 5~10 seconds prior to use.
4) Prepare sufficient MET Master Mix containing MET Reaction Mix A, MET Reaction Mix B and MET Enzyme Mix in a sterile tube according to the ratio in Table 3. Mix the MET Master Mix thoroughly by vortexing and centrifuge for 5~10 seconds.

Table 3 MET Master Mix

Content	Volume per test
MET Reaction Mix A	24.35 μL
MET Reaction Mix B	5 μL
MET Enzyme Mix	0.65 μL
Total	**30 μL**

Note:

- *Every PCR run must contain one PC and one No Template Control (NTC).*
- *The prepared mixtures should be used immediately, avoid prolonged storage.*
- *Due to the viscosity of the enzyme mix, pipet slowly to ensure all mix is completely dispensed from the tip.*
- *Pipet enzyme mix by placing the pipet tip just under the liquid surface to avoid the tip being coated in excess enzyme.*

5) Take out the sample RNA (we recommend use 20~500 ng/μL RNA) and nuclease-free water for NTC.
6) Prepare sufficient PCR tubes for PC, NTC and samples.
7) Dispense 30 μL MET Master Mix into each of the PCR tubes.
8) Add 10 μL nuclease-free water into the NTC tube, add 10 μL each of sample RNA into each sample tube, and add 10 μL MET Positive Control to the PC tube. Cap the PCR tubes.
9) Briefly centrifuge the PCR tubes to collect all liquid at the bottom of each PCR tube.
10) Place the PCR tubes into the real-time PCR instrument. A recommended plate layout is shown in Table 4.

Table 4 Plate Layout

Well	1	2	3
A	Sample 1	Sample 9	Sample 17
B	Sample 2	Sample 10	Sample 18
C	Sample 3	Sample 11	Sample 19
D	Sample 4	Sample 12	Sample 20
E	Sample 5	Sample 13	Sample 21
F	Sample 6	Sample 14	Sample 22
G	Sample 7	Sample 15	PC
H	Sample 8	Sample 16	NTC

11) Setup the PCR Protocol using the cycling parameters in Table 5.

Table 5 Cycling Parameters

Stage	Cycles	Temperature	Time	Data collection
1	1	42℃	5 min	/
		95℃	5 min	/
2	10	95℃	25 s	/
		64℃	20 s	/
		72℃	20 s	/
3	36	93℃	25 s	/
		60℃	35 s	FAM and HEX/VIC
		72℃	20 s	/

12) Start the PCR run immediately.

13) When the PCR run is finished, analyze the data according to the "Results Interpretation" procedures.

3. Results Interpretation

Before sample data analysis, the following items should be checked:

1) For NTC: The FAM Ct value should be ≥ **36**, if not, the data is *INVALID*. The sample should be retested.

2) For Positive Control: The FAM and HEX/VIC Ct values should be **< 25**, if not, the data is *INVALID*. The sample should be retested.

3) For the internal control HEX/VIC signal of each assay:

   a) The HEX/VIC Ct value should be ≤ **27**.

   b) If the HEX/VIC Ct value is > **27**, this indicates the RNA degradation or the presence of PCR inhibitors. The sample should be retested with increased or re-extracted RNA.

Analyze the mutation assay for each sample:

4) Check the FAM Ct value for each sample.

   a) If the mutant FAM Ct value is **> 27** or there is no amplification, the sample is determined as negative (no mutation detected) or under the LOD of the kit.

   b) If the mutant FAM Ct value is ≤ **27**, calculate the ΔCt value:

      i. **ΔCt value = Mutant FAM Ct value – Internal control HEX/VIC Ct value**.

      ii. If the ΔCt value is ≤ **6**, the sample is determined as positive (mutation detected).

      iii. If the ΔCt value is > **6**, the sample is determined as negative (no mutation detected) or under the LOD (Limit of Detection) of the kit.

Performance Characteristics

1) Limit of Detection:

   The kit allows detection of 25 copies/μL MET mutant plasmid DNA.

2) Specificity:

   The specificity of the kit was established by testing negative reference controls. The test gave negative results and negative concordance rate was 100%.

3) Accuracy:

   Accuracy of the kit was established by testing positive reference controls. The test gave positive results and positive concordance rate was 100%.

4) Precision:

   Precision of the kit was established by performing a certain mutant positive reference control for 10 repeats; CV (coefficient of variation) of ≤ 5%.

Limitations

1) The kit is to be used only by personnel specially trained with PCR techniques.

2) The kit has been validated for use with FFPE tumor tissue.

3) The kit can only detect the exon 14 skipping mutation in *MET* gene.

4) Reliable results are dependent on proper sample processing, transport, and storage.

5) The sample containing degraded RNA may affect the ability of the test to detect *MET* exon 14 skipping mutation.

6) Samples with negative result (No mutation detected) may harbor *MET* exon 14 skipping mutation not detected by this assay.

References

1) Herbst RS, Heymach JV, Lippman SM. Lung cancer. *The New England journal of medicine* 2008;359:1367-80.

2) Onozato R, Kosaka T, Kuwano H, Sekido Y, Yatabe Y, Mitsudomi T. Activation of MET by gene amplification or by splice mutations deleting the juxtamembrane domain in primary resected lung cancers. *Journal of Thoracic Oncology*, 2009;4:5-11.

3) Seo JS, Ju YS, Lee WC, et al. The transcriptional landscape and mutational profile of lung adenocarcinoma. *Genome res*. 2012;22:2109-19.

4) Comprehensive molecular profiling of lung adenocarcinoma. *Nature* 2014;511:543-50.

5) Frampton GM, Ali SM, Rosenzweig M, et al. Activation of MET via diverse exon 14 splicing alterations occurs in multiple tumor types and confers clinical sensitivity to MET inhibitors. *Cancer discovery* 2015;5:850-9.

6) Jenkins RW, Oxnard GR, Elkin S, Sullivan EK, Carter JL, Barbie DA. Response to Crizotinib in a Patient With Lung Adenocarcinoma Harboring a MET Splice Site Mutation. *Clinical lung cancer* 2015;16:e101-4.

7) Paik PK, Drilon A, Fan PD, et al. Response to MET inhibitors in patients with stage IV lung adenocarcinomas harboring MET mutations causing exon 14 skipping. *Cancer discovery* 2015;5:842-9.

8) Liu X, Jia Y, Stoopler MB, et al. Next-Generation Sequencing of Pulmonary Sarcomatoid Carcinoma Reveals High Frequency of Actionable MET Gene Mutations. Journal of clinical oncology : *official journal of the American Society of Clinical Oncology* 2016;34:794-802.

9) Awad MM, Oxnard GR, Jackman DM, et al. MET Exon 14 Mutations in Non-Small-Cell Lung Cancer Are Associated With Advanced Age and Stage-Dependent MET Genomic Amplification and c-Met Overexpression. *Journal of Clinical Oncology*, 2016;34:721-30.

Symbols