ROS1 Fusion Gene Detection Kit

Multiplex Fluorescence Polymerase Chain Reaction

Instruction for Use

For Research Use Only

Instruction Version: S2.0 Revision Date: November 2023

Product Name

ROS1 Fusion Gene Detection Kit (Multiplex Fluorescence Polymerase Chain Reaction)

Packing Specification

20 Tests/Kit

Intended Use

The kit uses multiplex fluorescence PCR amplification technology to qualitatively detect fusions in ROS1 gene (Table 1) from RNA isolated from paraffin-embedded (FFPE) tumor tissue samples from patients with non-small cell lung cancer (NSCLC). The test results are for research use only.

The proto-oncogene ROS1 encodes a transmembrane protein, which is a member of tyrosine kinase receptor superfamily and plays important role in cell growth and proliferation. When ROS1 gene fuses with SLC34A2, CD74, or other genes, the mutated fusion protein will auto-activate kinase domain and downstream signaling permanently, thus leads to cancer at last. The tyrosine kinase inhibitor of ROS1 exerts its function through competitively binding with the ROS1 kinase domain to block signaling transduction of ROS1 downstream pathways. Therefore, the sensitivity and accuracy of ROS1 fusion mutation detection has become a significant factor in aiding the treatment of patients with NSCLC.

Table 1. Types of ROS1 Gene Fusions

Reaction Tube	Exon of Fusion Genes	Exon of ROS1
ROS1-1	SLC34A2 exon4	Exon32
	SLC34A2 exon13 del2046	Exon32
	CD74 exon6	Exon32
	SDC4 exon2	Exon32
	SDC4 exon4	Exon32
ROS1-2	SLC34A2 exon4	Exon34
	SLC34A2 exon13 del2046	Exon34
	CD74 exon6	Exon34
	SDC4 exon2	Exon34
	SDC4 exon4	Exon34
	EZR exon10	Exon34
ROS1-3	TPM3 exon8	Exon35
	GOPC exon8	Exon35
	LRIG3 exon16	Exon35
	CCDC6 exon5	Exon35
	CLTC exon31	Exon35
ROS1-4	GOPC exon4	Exon36
	LIMA1 exon10	Exon36

Technological Principle

This kit uses multiplex fluorescence PCR technology to qualitatively detect various kinds of ROS1 gene fusion in RNA samples, collected from FFPE pathological tissue of NSCLC. This kit uses the sequence of designated fusion mutation sites and house-keeping gene GUSB as the template to design primers and fluorescent probes, and the target gene sequence length of each mutant is controlled within 150 bp. For product analysis, the use of fluorescently labeled probe real-time tracking analysis makes the detection method automatic. When analyzing the results, the FAM signal indicates the gene fusion mutation and the HEX (VIC) signal indicates the quality of sample RNA.

Kit Contents

The kit contains mixed enzyme (ROS1), positive control, and 4 reaction mix. For each sample tested, the reaction mix numbered 1 to 4 should be used at the same time. Each reaction mix contains specific primers and fluorescent probes of internal control genes as quality control of the reagents, RNA quality and operation.

Table 2. Kit Contents

Content Name	Components	Volume	Quantity
ROS1-1 Reaction Mix	Primers, probes, Mg^{2+}, dNTPs	700 μL	1 Tube
ROS1-2 Reaction Mix	Primers, probes, Mg^{2+}, dNTPs	700 μL	1 Tube
ROS1-3 Reaction Mix	Primers, probes, Mg^{2+}, dNTPs	700 μL	1 Tube
ROS1-4 Reaction Mix	Primers, probes, Mg^{2+}, dNTPs	700 μL	1 Tube
Mixed Enzyme (ROS1)	Reverse Transcriptase, Taq DNA polymerase, Uracil-DNA Glycosylase	160 μL	1 Tube
ROS1 Positive Control	Positive plasmid	100 μL	1 Tube

Note: The contents of different batches cannot be mixed.

Additional required Equipment and Materials

1. Commercialized nucleic acid extraction kit.
2. Nuclease-Free water (NTC).
3. Aerosol-barrier pipette tips.

Transportation, Stability and Storage

1. Storage Condition. Store the kit away from light at -15℃ to -25℃, valid for 9 months. Once opened, reagents can be stored in their original packaging at -15℃ to -25℃ until the stated expiration date shown on the packaging. Repeated thawing and freezing should be avoided. Do not exceed a maximum of 5 freeze-thaw cycles.
2. Transportation Condition. The kit should be transported at low temperature, with transporting time less than one week and transporting temperature lower than 25℃.
3. Check labels for production date and expiration date of the kit.

Compatible PCR Instruments

Stratagene Mx3000P™, ABI7500, SLAN-48P/96S, ABI StepOne Plus, etc.

1. For Stratagene Mx3000P™, FAM and HEX channel signal gain multiple is adjusted to 1.
2. For ABI instruments, the probe mode setting as follows: Reporter Dye: FAM, VIC; Quencher Dye: TAMRA; Passive Reference: NONE.

Specimen Material

1. Recommended sample types: FFPE tissues stored for no more than 2 years. The biopsies should be fixed with formalin and embedded in paraffin. For resection or surgical biopsies, the recommended tissue input is at least 2×5-micron sections. For coreneedle biopsies, the recommended tissue input is at least 10×5-micron sections.The tissue sample should contain at least 20% tumor cells, otherwise, the tissue samples should be macrodissected and enriched for tumor content.
2. Commercialized kit is recommended to extract RNA from the samples. Assess the quality of sample RNA with an microvolume ultraviolet-visible spectrophotometer, the ratio of OD_{260}/OD_{280} should be within the range of 1.8-2.3, the concentration is not less than 10 ng/μL. If the concentration of RNA is between 10-100 ng/μL, it is recommended to detect directly; if the concentration of RNA is greater than 100 ng/μL, RNA should be diluted to 100 ng/μL with Nuclease-Free Water before detection.
3. Proceed to sample detection or store the RNA at -15℃ to -25℃ for no more than 3 months. Freeze-thaw samples no more than 5 times.

Experimental Procedure

1. Reagent Preparation

   Take out the **ROS1 Reaction Mix** from the kit and put them on the ice. After the reaction mix melts, take 25 μL of reaction mixes and pack it into the 8-tube strips according to samples, and each 8-tube strip detects two samples, then cover the cap. Place 8-tube strips and

Mixed Enzyme (ROS1) on ice and transfer to the sample processing area detection of Positive Control (PC) and Negative Control (NTC, Nuclease-Free water) in each reaction/run is recommended.

2. Sample Processing
   (1) Commercialized kit is recommended to extract sample RNA, the concentration is 10-100 ng/μL, which is so called tested RNA.
   (2) Respectively add 6 μL **Mixed Enzyme (ROS1)** to 20 μL of the tested RNA, PC and NTC, vortex slightly to mix, then pulse centrifuge, which is so called amplification template.
   (3) Gently remove the cap of 8-tube strip, sequentially add 5.2 μL of the RNA/PC/NTC templates into tubes of each strip, (that is,4 μL sample and 1.2 μL **Mixed Enzyme (ROS1)** are added to each reaction tube), cover the cap carefully and transfer to the amplification detection area.

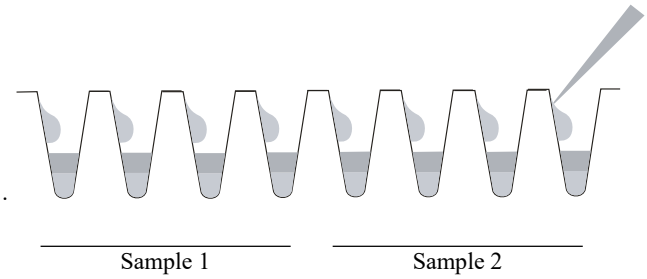

Figure 1. The 8-Tube Strip Sampling Diagram

3. Amplification
   (1) Centrifuge the 8-tube strips for 10 seconds to collect templates.
   (2) Load the 8-tube strips into the real-time PCR instrument; refer to Table 3 for overall arrangement if necessary.

Table 3. Suggested Overall Arrangement

No.	Assay	1	2	3	4	5	6	7	8	9	10	11	12
1	ROS1-1	Sample1	Sample3	Sample5	Sample7	Sample9	Sample11	Sample13	Sample15	Sample17	Sample19	Sample21	PC
2	ROS1-2	Sample1	Sample3	Sample5	Sample7	Sample9	Sample11	Sample13	Sample15	Sample17	Sample19	Sample21	PC
3	ROS1-3	Sample1	Sample3	Sample5	Sample7	Sample9	Sample11	Sample13	Sample15	Sample17	Sample19	Sample21	PC
4	ROS1-4	Sample1	Sample3	Sample5	Sample7	Sample9	Sample11	Sample13	Sample15	Sample17	Sample19	Sample21	PC
5	ROS1-1	Sample2	Sample4	Sample6	Sample8	Sample10	Sample12	Sample14	Sample16	Sample18	Sample20	Sample22	NTC
6	ROS1-2	Sample2	Sample4	Sample6	Sample8	Sample10	Sample12	Sample14	Sample16	Sample18	Sample20	Sample22	NTC
7	ROS1-3	Sample2	Sample4	Sample6	Sample8	Sample10	Sample12	Sample14	Sample16	Sample18	Sample20	Sample22	NTC
8	ROS1-4	Sample2	Sample4	Sample6	Sample8	Sample10	Sample12	Sample14	Sample16	Sample18	Sample20	Sample22	NTC

   (3) Set and run the amplification program as shown in Figure 2.

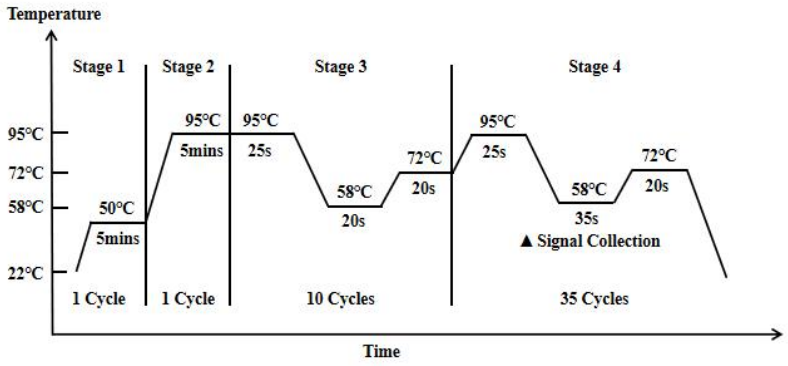

Figure 2. PCR Amplification Procedure

   (4) Handle the strips properly after experiment; do not remove the caps in case contamination.

Data Analysis

1. The positive judgment value of this kit is determined as 30 with the assist of ROC curve method.
2. Result Judgment
   (1) When there are no FAM signal amplification curve rises, a negative call or lower than the detection limit of the kit is returned.
   (2) When the FAM signal amplification curve of any of the four reaction tubes of the sample rises and the Ct is less than 30, a positive call is returned.
   (3) When the FAM signal amplification curve of any of the four reaction tubes of the sample rises but the Ct is greater than or equals to 30, increase concentration and re-detect. When the Ct value of the retest result is less than 30, a positive call is returned, otherwise, a negative call or lower than the detection limit of the kit is returned.

Interpretation of Results

1. NTC: There should be no amplification curves of FAM or HEX (VIC) in each NTC reaction tube, or else, call the result invalid, recommend to test again.
2. PC: The FAM Ct and HEX (VIC) Ct of all PC reaction tubes should be less than 24; or else, call the result invalid, recommend to test again.
3. Internal Control: The HEX (VIC) Ct of internal control (tube 4 or tube 8) should be less than 26, which must be qualified before proceeding to further analysis; if the HEX(VIC) Ct is greater than or equals to 26, that indicates insufficient RNA amount or the sample RNA was contaminated with PCR inhibitor, in this case, it is recommended to re-extract sample RNA for a new detection.

Limitations of the Kit

1. The test results of this kit are for scientific research reference only.
2. Negative results could not exclude the existence of ROS1 gene fusion completely; cases like inadequate tumor cells, RNA degradation, or insufficient RNA amount may lead to negative results as well.
3. Different sampling locations may lead to diverse outcomes due to the heterogeneity of tumor tissues/cells.
4. Situations that may result in false negative or false positive result include but not limit to unreasonable sample collection, transportation, improper experimental operations or environment.
5. The kit is only intended for the qualitative detection of 18 specific gene fusions of ROS1 gene in patients with NSCLC.
6. The kit is only applicable with the stated sample types and detection system, including specified instruments, RNA extraction kit and analytical assay.

Performance Characteristics

1. The kit should be of neat appearance, clear labels, and of no leakage; when unfrozen, the reagents shall be clear, without precipitate.
2. The consistency rates of both positive and negative reference materials are 100%.
3. The kit allows the detection as low as 100 copies of ROS1 gene fusion in 40 ng RNA samples.
4. There's no nonspecific product with up to 400 ng wild-type RNA sample.
5. For 10 repetitive times detection of the designated sample, the Ct values of FAM and HEX (VIC) channel should be less than 24, and the coefficient of variation (CV, %) of Ct values should be less than 5%.

Warnings and Precautions

1. Please read the instruction carefully in prior to the use of the kit.
2. Avoid repetitively freezing and thawing reagents in the kit.
3. The results of this kit will be affected by the source, the process of collection, quality, condition of transport, pre-treatment of the sample, as well as the quality of the extracted RNA, model of fluorescence quantitative PCR instrument, operation environment, and the current technological limitation of molecular biology. The factors and variables mentioned above would lead to false positive or false negative test results. Users must be aware of the potential errors and accuracy limitations that may exist during the process of detection.
4. The quality of RNA is crucial, commercialized RNA extraction kit is recommended, and the quality control of RNA should be performed after extraction; proceed to sample detection immediately or store sample RNA properly at -15℃ to -25℃. It can be stored below -70℃ for no more than 12 months in order to extend the storage period of the sample.
5. Do not substitute any content of the kit; do not mix contents of different batches.

6. Pay special attention to the use of positive control to prevent contamination of reagents or resulting in false positive results.
7. Be cautious of contamination from external RNA. Segregate areas of reagent preparation and sample processing; use dedicated pipettes and tips for reagent preparation and template addition, respectively.
8. Sterilize the environment and pipettes with 10% hypochlorous acid, 75% ethyl alcohol, or UV radiation.
9. All the reagents in use have potential hazard. It is recommended wearing proper protective suits and gloves. For first-use of this kit, you may receive training by our technical supports.
10. All samples including positive control in the kit should be considered as potential infectious substances which should be handled carefully.

Symbols

Symbol	Symbol Definition
	Indicates the need for the user to consult the instructions for use.
	Indicates the date when the medical device was manufactured.
	Indicates the manufacturer's batch code so that the batch or lot can be identified.
	Indicates the temperature limits to which the medical device can be safely exposed.
	Indicates the date after which the medical device is not to be used.
	This is the correct upright position of the distribution packages for transport or storage.
	Indicates a medical device that needs to be protected from moisture.
	Indicates a medical device that needs protection from light sources.
	Indicates the medical device manufacturer.

References

1. Davies K D, Doebele R C. Molecular pathways: ROS1 fusion proteins in cancer[J]. Clinical Cancer Research An Official Journal of the American Association for Cancer Research, 2013, 19(15):4040-4045.
2. Matsuura S, Shinmura K, Kamo T, et al. CD74-ROS1 fusion transcripts in resected non-small cell lung carcinoma[J]. Oncology Reports, 2013, 30(4):1675-1680.
3. Bergethon K, Shaw A T, Ou S H, et al. ROS1 rearrangements define a unique molecular class of lung cancers[J]. Journal of Clinical Oncology, 2012, 30(8):863-870.
4. Rikova K, Guo A, Zeng Q, et al. Global Survey of Phosphotyrosine Signaling Identifies Oncogenic Kinases in Lung Cancer[J]. Cell, 2007, 131(6):1190-1203.
5. Takeuchi K, Soda M, Togashi Y, et al. RET, ROS1 and ALK fusions in lung cancer.[J]. Nature Medicine, 2012, 18(3):378-381.

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