PAP-ARMS®

Human NTRK Genes Fusion Detection Kit

Multiplex Fluorescence Polymerase Chain Reaction

Instruction for Use

Product Name

Human NTRK Genes Fusion Detection Kit (Multiplex Fluorescence Polymerase Chain Reaction)

Packing Specification

20 Tests/Kit

Intended Use

This kit uses multiplex fluorescence PCR amplification technology to qualitatively detect NTRK1 (NM_001012331.1) NTRK2（NM_006180.4） and NTRK3（NM_001012338.2） (Refer to Table 1)gene fusion mutations in RNA samples extracted from paraffin embedded tissue sections or fresh tissues from cancer patients. The detection results are only for clinical reference, which are not the only basis for individualized treatment of patients. In clinical application, comprehensive judgment should be made in combination with the patient's condition, drug indications, treatment response and other laboratory test indicators.

NTRK is a gene encoding TRK. There are three genes, including NTRK1, NTRK2, NTRK3, which are located in different segments of chromosome 1q22, 9q21 and 15q25. The corresponding encoded proteins are called TRKA, TRKB and TRKC. The tyrosine kinase region is located at the 3'end of NTRK gene. When the partner gene is fused with NTRK gene, the TRK fusion protein will remain active and trigger a signal cascade reaction, which will drive and promote the diffusion and growth of TRK fusion tumor. Among them, the fusion frequency of NTRK1 and NTRK3 genes is higher than NTRK2.

Table 1 NTRK Fusion Gene Types

Reaction Tube	Detection Channel	Splicing Gene and Exon	NTRK1/2 gene exon
NTRK-1	FAM	LMNA E2	NTRK1 E10
		LMNA E10	NTRK1 E10
		LMNA E11	NTRK1 E10
		SQSTM1 E5	NTRK1 E9
		TPM3 E8	NTRK1 E9
	HEX（VIC）	NACC2 E4	NTRK2 E12
		SQSTM1 E5	NTRK2 E13
		VCL E16	NTRK2 E13
NTRK-2	FAM	TFG E5	NTRK1 E9
		TFG E6	NTRK1 E9
		NFASC E21	NTRK1 E9
		CD74 E8	NTRK1 E9
		TPR E21	NTRK1 E9
		TPR E10	NTRK1 E9
		IRF2BP2 E1	NTRK1 E9
		BCAN E12	NTRK1 E10
		BCAN E13	NTRK1 E9
		LMNA E5	NTRK1 E9
NTRK-3	FAM	MPRIP E14	NTRK1 E11
		MPRIP E18	NTRK1 E11
		MPRIP E21	NTRK1 E11
		LMNA E6	NTRK1 E11
		TPM3 E8	NTRK1 E11
		SSBP1 E12	NTRK1 E11
	HEX（VIC）	STRN E3	NTRK2 E16

		QKI E6	NTRK2 E16
NTRK-4	FAM	ETV6 E4	NTRK3 E14
		ETV6 E5	NTRK3 E15
		ETV6 E4	NTRK3 E15
		ETV6 E5	NTRK3 E14
		EML4 E2	NTRK3 E15
		TFG E6	NTRK3 E14

Technological Principles

The reagent detects the RNA extracted from FFPE tissue sections or fresh tissues from solid tumor patients. The sample RNA was reverse transcribed into cDNA under reverse transcriptase, and the gene fusion status of NTRK gene was qualitatively detected on the real-time PCR instrument by using multiple fluorescence PCR amplification technology. Primers and fluorescent probes were designed with the fusion mutation sequence as the template. The length of the target gene sequence of each mutation was controlled within 150 bp. The target gene sequence of the internal standard is a conserved sequence on the human housekeeper ACTB gene. In terms of product analysis, fluorescence-labeled probe real-time tracking analysis technology is used to automate the detection method. The NTRK gene fusion status is indicated by FAM or HEX(VIC) signal, and the quality control of reagent, cDNA quality, and operation is indicated by the reaction tubes 4 or 8 HEX (VIC) signal.

Kit Contents

The kit contains reverse transcription reaction components, Taq polymerase, positive control, and reaction mixes (Table 2). The reaction mixes contain specific primers, fluorescent probes, dNTPs, magnesium chloride, ammonium sulfate and potassium chloride. Primer probes of internal control genes are added in NTRK-4 reaction mix as the quality control of reagent, cDNA quality, and operation.

Table 2 Kit Contents

Content Name	Volume	Quantity
NTRK-1 Reaction Mix	700 μL	1 tube
NTRK-2 Reaction Mix	700 μL	1 tube
NTRK-3 Reaction Mix	700 μL	1 tube
NTRK-4 Reaction Mix	700 μL	1 tube
NTRK Taq Polymerase	30 μL	1 tube
NTRK Positive Control	40 μL	1 tube
Reverse Transcription Premix	300 μL	1 tube
Random Primer	25 μL	1 tube
Reverse Transcriptase	25 μL	1 tube
Nuclease-Free Water	200 μL	1 tube

Note: The contents of different batches cannot be mixed.

Equipment and Reagents Required

1. Microvolume UV-Vis spectrophotomete;
2. Commercial nucleic acid extraction kit;
3. DNase-free and RNase-free pipettes and tips,

Transportation, Stability and Storage

1. Storage Condition. Store the kit away from light at -20±5 ℃, valid for 9 months. Once opened, the kit is stable at -20±5 ℃ until the stated expiration date. Do not use the reagents after 5 freezing-thawing cycles.
2. Transportation Condition. The kit should be transported in foam cases with ice bags, 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.

Applicable Instruments

Stratagene Mx3000P™, Mx3005P, ABI7500, ABI7300, ABI7900, LightCycler 480, ABI 7500 Fast, ABI StepOne Plus.

1. For ABI instruments, define targets and passive reference as follows: Reporter Dye: FAM, VIC; Quencher Dye: TAMRA; Passive Reference: NONE.
2. For Stratagene Mx3000P™/Mx3005P™, if there's low net fluorescence signal (dR) but high background signal (R), reduce the filter set gain multiplier properly.

Specimen Material

1. Recommended sample types: FFPE tissue section, fresh tissue. After pathological evaluation, it should contain at least 30% of tumor cells.
2. It is recommended to use a commercial kit to extract RNA. Assess the quality of sample RNA with an ultraviolet spectrophotometer, the concentration should be ≥ 10 ng/μL, the ratio of OD_{260}/OD_{280} should be within the range of 1.8 - 2.3. If the RNA concentration and purity do not meet the requirements of the reverse transcription kit, resampling or expanding the sample size before RNA extraction.
3. It is recommended to reverse transcribe the extracted RNA immediately, or store it below -70℃ for no more than 6 months, and avoid repeated freezing and thawing during the period
4. The preservation time of FFPE pathological tissue or section samples shall not exceed 2 years.

Experimental Procedure

1. Reagent preparation (Reagent Preparation Area)
   a) Transfer the needed PCR reaction tubes without DNase and RNase, add 1 μL Random Primer respectively, place the tubes in the ice box, then move them to the sample processing area for use.
   b) Transfer another RNase/DNase-free centrifuge tube, and prepare a reverse transcription reaction solution in equal proportion according to the number of samples. The reverse transcription reaction solution of each sample is:1 μL Reverse Transcriptase and 14 μL Reverse Transcription Premix. Place it in an ice box and move it to the sample processing area for use.
   c) Remove the reaction mixes and place them on the ice box to thaw, and then dispack into 8-tube strip, 25μL per serving. Place them on ice before transferring to the sample processing area; It is recommended to analyze samples, positive control (PC), and negative control (NTC, purified water) at the same time in each PCR reaction.
2. Sample Processing (Sample Processing Area)
   a) Using commercial nucleic acid extraction reagent to extract RNA.
   b) Add 4 μL sample RNA (the total amount should not exceed 2 μg, supplement with Nuclease-Free Water when the volume is less than 4 μL) to the PCR reaction tube pre-loaded with 1 μL Random Primer. Take an ice bath for 5 min after incubating at 70℃ for 5 min. Centrifugate briefly, then add 15 μL of the above reverse transcription reaction solution. Mix well and perform the process of reverse transcription referring to reaction condition of table 3;

Table 3 Reverse Transcription Reaction Conditions

Temperature	Time
25 ℃	5 min
42 ℃	60 min
70 ℃	15 min
4 ℃	∞

   c) Transfer the PCR reaction tube, centrifuge briefly, add 1 μL NTRK Taq Polymerase and mix well, which is the amplification template;

d) Gently remove the cap of the NTRK 8-tube reaction strip (place in the ice box), and add 5 μL of the above amplification templates to each tube of the NTRK 8-tube strip according to the example in the figure below. Each reaction strip detects two samples. It is recommended to analyze the samples, positive control (PC) and negative control (NTC) at the same time in each PCR reaction. Carefully cover the 8-tube reaction strip, and move to the amplification detection area.

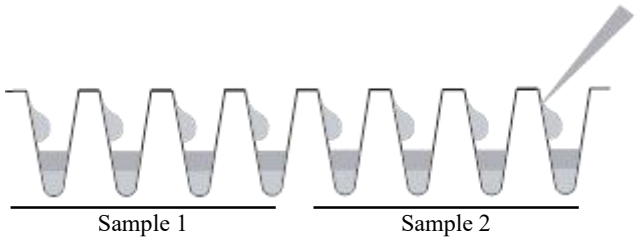

Fig. 1 Schematic Diagram of 8-tube Strip Sampling

3. Detection (Amplification Area)
   a) Rapid centrifugation of 8-tube strip for 10 seconds;
   b) Place the reaction strip into the real-time PCR instrument;
   c) Open the instrument window and set the amplification program according to figure 3;

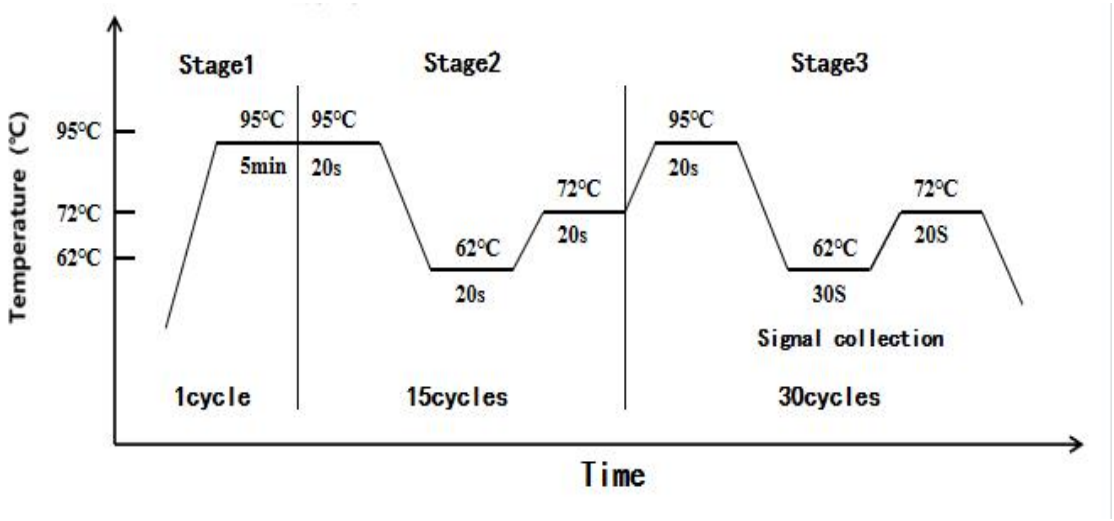

Fig. 2 PCR Amplification Procedure

   d) Run real-time PCR and save the file;
   e) Handle the stripes properly after the experiment; do not remove the caps in case of contamination.

Positive Judgment Value

If any of the FAM signals from the four reaction tubes or the HEX signal of NTRK-1 and NTRK-3 reaction tubes rises, the sample is judged as positive for gene fusion. Otherwise, it is judged as negative or below the detection limit.

Interpretation of Results

1. The FAM and HEX (VIC) signals of the negative control shall have no curve rise. If the FAM or HEX (VIC) signal curve of any negative control tube rises, the experimental result is invalid, it is recommended to retest.
2. The FAM and HEX (VIC) signals of positive control shall have a curve rise; the Ct value of positive control shall be ≤ 25. If the Ct value of FAM or HEX (VIC) signal of any positive control tube is higher than 25, the experimental result is invalid, it is recommended to retest.
3. The HEX (VIC) signal Ct value of the internal control tube (tube 4 or tube 8) shall be ≤ 20, the next analysis can be carried out while the quality control is qualified. If the Ct value of the HEX (VIC) signal is > 20, it indicates that the added cDNA template contains a PCR inhibitor or the concentration is too low, so it is necessary to re-extract RNA and reverse transcription before re-detection.

Limitation of the Kit

1. The results are only for research reference.

2. Negative results can not exclude the NTRK fusion mutation. less tumor cells in the sample, excessive nucleic acid degradation, or lower concentration of target gene in the amplification reaction system can also cause negative results.
3. Due to the heterogeneity of tumor tissues (cells), different results may be obtained by sampling different parts.
4. The unreasonable sample collection, transportation, treatment, improper operation and experimental environment may lead to false negative or false positive results.
5. The kit is only applicable with the stated sample types and detection system, including specified instruments, DNA extraction kit and analytical assay.

Physical Performance

1. The kit should be of neat appearance, have clear labels, and of no leakage; When unfrozen, the reagents shall be clear, without sediments.
2. The consistency rates of positive control are 100%.
3. The consistency rates of negative control are 100%.
4. Fusion mutations as low as 100 copies in RNA samples can be detected.
5. Repeat the test for the same precision reference for 10 times, the Ct value of FAM and HEX channels shall be less than 25, and the coefficient of variation (CV%) of Ct value shall be less than 10%.

Precautions and Warning

1. Please read this manual carefully before the experiment.
2. Avoid repeatedly freezing and thawing the reagent.
3. The results of this kit will be affected by the source of the sample, sample collection process, sample quality, sample transportation conditions, sample pretreatment and other factors. At the same time, it will also be limited by RNA extraction quality, fluorescence quantitative PCR instrument model, operating environment and the limitations of current molecular biology technology, which may lead to false positive or negative results. Users should understand the potential errors and limitations of accuracy that may exist in the detection process.
4. It is very important to detect the quality of RNA. It is recommended to use a commercial RNA extraction kit to extract RNA. After RNA extraction, quality control should be carried out to determine the extraction quality. If experiments cannot be carried out immediately, the extracted RNA should be stored below - 70℃.
5. All reagents in this kit have been specially prepared, any replacement of any reagent may affect the effect; Components of the kits with different batch numbers shall not be mixed with each other.
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 RNase to the reagent. Segregate areas for 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, or 75% ethyl alcohol, or UV radiation.
9. All the reagents in use have potential hazard. Only people who have work permit for PCR laboratories are allowed to use this kit. It is suggested to wear proper protective suit 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 potential infectious substances. They should be handled carefully.

Notes

Symbol	Legend
	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.
	Indicates the authorized representative in the European Community/European Union.
	The product meets the basic requirements of European in vitro diagnostic medical devices directive 98/79/EC.

Reference

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