Human Breast Cancer 21 Genes Expression Detection Kit

Multiplex Fluorescence Polymerase Chain Reaction

Instruction for Use

Product Name

Human Breast Cancer 21 Genes Expression Detection Kit (Multiplex Fluorescence Polymerase Chain Reaction)

Packing Specification

6 Tests/Kit

Intended Use

This kit uses multiplex fluorescence PCR amplification technology to extract RNA from paraffin-embedded tissue sections from HR-positive and HER2-negative patients with early breast cancer to qualitatively detect RNA expression level of 21 specific genes, and to evaluate the chemotherapy benefit and risk of recurrence in breast cancer patients. The test results are for research reference only.

The kit is intended for detecting the expression level of 21 specific genes (Table 1), of which 16 genes are involved in cancer cell proliferation, invasion, HER2 and estrogen related signaling, 5 genes in the kit as references. By detecting expression level of 21 genes, predict the recurrence index of breast cancer, judge the benefit of patient receiving chemotherapy and distinguish the characteristic of tumor through study the association between the 21 genes.

Table 1 Detection of genes by Kit

No.	21-Gene 8-Tube Strips 1		21-Gene 8-Tube Strips 2		21-Gene 8-Tube Strips 3	
	Genes	Group	Genes	Group	Genes	Group
1	GRB7	HER2	MYBL2	proliferation	β-actin	references
2	HER-2		Cyclin B1		GAPDH	
3	ER	estrogen	STK15		RPLPO	
4	PR		Cathepsin L2	invasion	GUS	
5	BCL2		Stromelysin 3		TFRC	
6	SCUBE2		CD68	others	/	/
7	Survivin	proliferation	GSTM1		/	/
8	Ki-67		BAG1		/	/

Technological Principles

This kit uses multiplex fluorescence PCR technology to detect the expression level of 21 specific genes in the sample, and the kit uses sequence of the cDNA sequence of designated genes as a template to design ARMS primers and fluorescent probes. For product analysis, the use of fluorescently labeled probe real-time tracking analysis makes the detection method automatic.

Kit Contents

The kit contains 21 kinds of reaction reagents, which were pre-loaded in 8-tube strips. Each PCR reaction tube contains specific primers, fluorescent probes, dNTPs, $MgCl_2$, etc. There are three kinds of 8-tube strips (Table 2), and one sample is tested by 8-tube strips 1,2 and 3 together.

Table2. Kit Contents

Content Name	Components	Volume	Quantity
21-Gene 8-Tube Strips 1	Primers, probes, Mg^{2+}, dNTPs	25 μL	8 strips
21-Gene 8-Tube Strips 2	Primers, probes, Mg^{2+}, dNTPs	25 μL	8 strips
21-Gene 8-Tube Strips 3	Primers, probes, Mg^{2+}, dNTPs	25 μL	8 strips
Taq Polymerase (21-Gene)	Taq DNA polymerase	60 μL	1 Tube
Reverse Transcription Enzyme	Reverse Transcription Enzyme	8 μL	1 Tube

Reverse Transcription Reaction Solution	Primers, Mg^{2+}, dNTPs	15 μL	1 Tube
21-Gene Positive Control	Positive plasmid DNA	250 μL	1 Tube
Nuclease-Free Water	Nuclease-Free Water	1 mL	1 Tube

Note: The contents of different batches cannot be mixed.

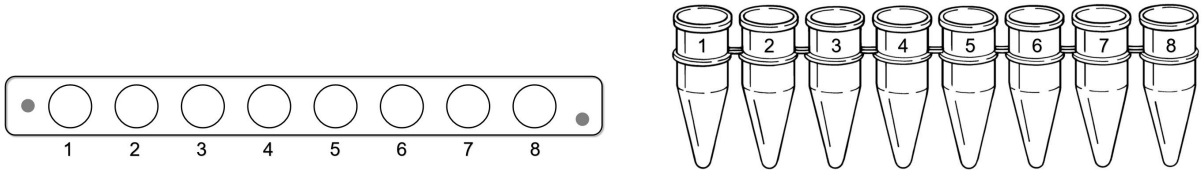

Figure 1. Tube Sequence of 8-Tube Strip

Note: The reaction solution has been pre-loaded in 8-tube strips, as shown in Figure 1, there are two different models on the left and right, which are 1, 2, 3, 4, 5, 6, 7 and 8 tubes from left to right.

Materials and Equipment Required but not Provided

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 -25℃ to -15℃, valid for 9 months. Once opened, the kit is stable at -25℃ to -15℃ until the stated expiration date. Freeze-thaw reagents no more than 5 times.
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. Applicable population: HR-positive and HER2-negative early breast cancer patients, divided into two subtypes: hormone receptor positive, HER2 negative and lymph node negative stage I or II invasive breast cancer patients; postmenopausal, hormone receptor positive, HER2 negative and lymph node positive invasive breast cancer patients.
3. 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 70 ng/μL. Once the RNA quality or quantity was not in conformity with the above requirements, re-extract RNA with new and/or larger input.
4. Proceed to reverse transcribe or store the RNA at -20℃ for no more than 3 months. Freeze-thaw samples no more than 5 times.

Experimental Procedure

1. Template Preparation

a) Commercialized kit is recommended to extract RNA; prepare the reverse transcription reaction mix with the contents in Table 3;The total amount of RNA does not exceed 1000 ng, and filled with Nuclease-Free water when the volume is less than 7 μL.

Table 3. RNA Reverse Transcription Reaction Mix

Content Name	Volume/sample
Reverse Transcription Enzyme	1 μL
Reverse Transcription Reaction Solution	2 μL
Total RNA (≤1000 ng)	7 μL

b) Load the prepeared reaction mix into the PCR instrument; set and run the program shown in Table 4; the cDNA derived from reverse transcription can be stored at 10°C overnight or at -25°C to -15°C for long conservation.

Table 4. RNA Reverse Transcription Procedure

Temperature	Volume
42°C	30 mins
85°C	5 mins
10°C	∞

2. Reagent Preparation

Prepare 8-tube strips and Taq Polymerase (21-Gene) per the number of samples; briefly centrifuge the strips and Taq polymerase; place them on ice before transferring to the sample processing area; detection of 21-Gene Positive Control (PC) and Negative Control (NTC, Nuclease-Free water) in each reaction/run is recommended.

3. Samples Processing

a) Dilute the cDNA sample that obtained by reverse transcription with 100 μL Nuclease-Free water, vortex sightly to mix, which is so called tested sample template;

b) Respectively pipet 6.6 μL Taq Polymerase (21-Gene) to 110 μL of the tested cDNA sample, PC, and NTC,vortex slightly to mix, then pulse centrifuge, which is so called amplification template;

c) Gently remove the cap of 8-tube strips, sequentially pipet 5 μL of the templates into tubes of each strip, cover the cap carefully.

4. Amplification

a) Centrifuge the 8-tube strips for 10 seconds to collect templates;

b) Load the 8-tube strips into the real-time PCR instrument; refer to Table 5 for overall arrangement if necessary;

Table 5. Suggested Overall Arrangement

No.	1	2	3	4	5	6	7	8	9	10	11	12
1	Sample1	Sample1	Sample1	Sample2	Sample2	Sample2	PC	PC	PC	NTC	NTC	NTC
2	Sample1	Sample1	Sample1	Sample2	Sample2	Sample2	PC	PC	PC	NTC	NTC	NTC
3	Sample1	Sample1	Sample1	Sample2	Sample2	Sample2	PC	PC	PC	NTC	NTC	NTC
4	Sample1	Sample1	Sample1	Sample2	Sample2	Sample2	PC	PC	PC	NTC	NTC	NTC
5	Sample1	Sample1	Sample1	Sample2	Sample2	Sample2	PC	PC	PC	NTC	NTC	NTC
6	Sample1	Sample1	-	Sample2	Sample2	-	PC	PC	-	NTC	NTC	-
7	Sample1	Sample1	-	Sample2	Sample2	-	PC	PC	-	NTC	NTC	-
8	Sample1	Sample1	-	Sample2	Sample2	-	PC	PC	-	NTC	NTC	-

c) Set and run the amplification program as shown in Figure 2;

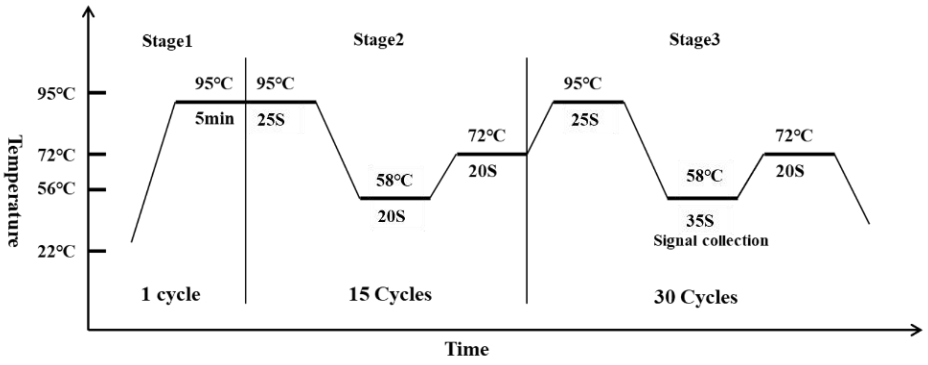

Figure 2. PCR Amplification Procedure

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

Interpretation of Results

1. Quality Control of the Results
   a) NTC: There should be no amplification curves of FAM and HEX in NTC reaction tube; or else, call the result invalid.
   b) PC: The FAM Ct of PC is generally less than 25, which can fluctuate with the threshold setting of different instruments; or else, call the result invalid.
2. Result Judgment
   a) Ct value: Provided by the instrument software or by determining the threshold fluorescence of actual amplification curve.
   b) Result judgment:
      I Obtain Ct values of 21 genes from instrument;
      II Input the Ct values of 21 genes to matched software, obtain the RS value, and judge the risk rank according to Table 6.

Table 6. Result Judgment

Patient Group	Risk Rank	RS Value (0-100)
Patients with premenopausal, HR-positive, HER2-negative, T1b/c-2, pN0	Low Risk	RS<16
	Intermediate Risk	16≤RS<26
	High Risk	RS≥26
Patients with premenopausal, HR-positive, HER2-negative, pT1-3, pN1	Low Risk	RS<26
	High Risk	RS≥26
Patients with postmenopausal, HR-positive, HER2-negative	High Risk	RS<26
	High Risk	RS≥26

Limitation of the Kit

1. The kit is indicated only for use as an aid in the clinical reference. Determinants such as, but not limited to patients' condition, drug indications, therapeutic response, and other laboratory detection indexes should also be considered before making comprehensive judgments.
2. Different sampling locations may lead to diverse outcomes due to the heterogeneity of tumor tissues/cells.
3. 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.
4. The kit is only applicable with the stated sample types and detection system, including specified instruments, RNA extraction kit and analytical assay.

Precautions and Warning

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. Perform quality control of RNA after extraction; proceed to quality control to determine the extraction quality and reverse transcription immediately or store sample RNA properly at -20℃. It can be stored below -70℃ for no more than 12 months in order to extend the storage period of the sample.
4. Do not substitute any content of the kit; do not mix contents of different batches.
5. Pay special attention to the use of positive control to prevent contamination of reagents or resulting in false positive results.
6. Sterilize the environment and pipettes with 10% hypochlorous acid, 75% ethyl alcohol, or UV radiation.
7. All the reagents in use have potential hazard. It is recommended wearing proper protective suit and gloves. For first-use of this kit, you may receive training by our technical supports.
8. 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.

Reference

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4. Musgrove E A, Sutherland R L. Biological determinants of endocrine resistance in breast cancer[J]. Nature Reviews Cancer, 2009, 9(9):631-43.
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6. Esteva F J, Hortobagyi G N. Prognostic molecular markers in early breast cancer.[J]. Breast Cancer Research, 2004, 6(3):109-18.
7. NCCN Clinical Practice Guidelines in Oncology for NSCLC.2013.

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