Human Y-chromosome TSPY Gene Probe qPCR Kit V2.0 (Lyophilized Powder) Manual V0.53

Catalog Number: 17-001-Y-TSPYv2, 48 T

Product Description

Skygen's Human Y-chromosome TSPY Gene Probe qPCR Kit V2.0 (Lyophilized Powder) is designed for both quantitative and qualitative detection of Human Y-chromosome TSPY Gene-specific DNA in a real-time PCR test using hydrolysis probes. In the amplification process, Taq DNA polymerase amplifies the DNA using two primer-probe sets. One targets a Human Y-chromosome TSPY Gene-specific DNA region and the other targets an endogenous Internal Control (IC) DNA region. At each cycle of the PCR, the 5' nuclease activity of Taq DNA polymerase degrades the bound probes, releasing the quenched Human Y-chromosome TSPY Gene-specific FAM signal and IC-specific Cy5 signal. The increase in FAM and Cy5 fluorescence measures the Human Y-chromosome TSPY Gene-specific and IC-specific DNA amplifications, respectively. At a point where the fluorescence signal is confidently detected over the background signal, a Ct value can be determined. This Ct parameter can be used to quantitatively evaluate Human Y-chromosome TSPY Gene in samples. Skygen's Human Y-chromosome TSPY Gene Probe qPCR Kit V2.0 (Lyophilized Powder) has the following features:

1. One-tube PCR, minimized carry-over contamination.
2. Supplied as lyophilized powder, transported and stored at ambient temperature.
3. High sensitivity, with an analytical LOD at 100 copies/reaction.
4. High specificity, no cross-reaction with DNA from other organisms.
5. Amplification positive control is provided in the kit.
6. False positive result can be ruled out using the endogenous IC.
7. Compatible with various DNA purification methods.
8. Suitable for both quantitative and qualitative test. For quantitative test, the linear range is at least 5 orders of magnitude.
9. Enough for 48 reactions, 25 μL per reaction.
10. Research use only.

Input Sample Requirements

- Highly suggest to use DNA purified with Qiagen QIAamp DNA Mini Kit or Thermo Fisher MagMAX Nucleic Acid Isolation Kit.

Kit Components

Component Name	Product Number	Size	Vial	Lid Color
Probe qPCR MasterMix Lyophilized Powder	250804	8 T × 6	Sealed 8-Well Strips	Natural
Template Dilution Buffer Powder	180701v2	48 T	1.5 mL	Green
Human Y-chromosome TSPY Gene and IC Primer-Probe Mix Lyophilized Powder	yp17-001-Y-TSPY	48 T	0.5 mL Brown	Brown
Human Y-chromosome TSPY Gene Positive Control Lyophilized Powder	pc001-Y-TSPYv2	48 T	0.5 mL	Yellow
Ultrapure Water	210806	1 mL	1.5 mL	Blue
Manual	sc17-001-Y-TSPYv2	1 Set	NA	NA

Storage and Handling

Reagents are shipped at ambient temperature and should be stored at room temperature upon receipt. The kit is stable for at least one year from date of receipt. After reconstitution of the lyophilized components, repeated thawing and freezing of the solutions should be avoided. All solutions must be thawed at room temperature before use, placed on ice during use and stored at -20°C after use. Thawed components should be mixed well by gentle vortex or pipetting before reaction

setup.

Materials and Equipment Required but not Supplied in the Kit

Unless otherwise indicated, all materials are available through major laboratory suppliers.

Class	Items
General laboratory equipment and consumables	Benchtop microcentrifuge, micropipettors, vortex mixer, , disposable powder-free gloves, sterile pipette tips with filters, RNase-free water, ultrapure water
Kit-specific laboratory equipment and consumables	Manual or automated DNA purification kits & equipments, real-time fluorescent PCR instruments with FAM channel and Cy5 channel and consumables, PCR reaction preparation station, known positive sample and negative sample as controls

Procedural Guidelines

- Use purified DNA as starting materials.
- Perform all steps at room temperature (20–25℃).
- Use sterile, disposable nuclease-free pipette tips and microtubes.
- Wear disposable gloves while handling reagents and DNA samples.

Procedures

Section 1: Prepare 1E1 - 1E6 copies/μL Serial Dilutions for Standard Curve (SDSC).

Note: Since the concentration of the Human Y-chromosome TSPY Gene Positive Control is very high, the following dilution operations must be carried out in an independent area to avoid its possible contamination of the samples or the kit components.

1. When using the kit for the first time, place the tube on ice. Add 1 mL RNase-free water into the vial that contains the Template Dilution Buffer Powder. Vortex the vial for 10 seconds and spin it down for 10 seconds. The solution thus obtained is the Template Dilution Buffer. Add 50 μL Template Dilution Buffer into the vial that contains the Human Y-chromosome TSPY Gene Positive Control Lyophilized Powder. Vortex the vial for 10 seconds and spin it down for 10 seconds. The solution thus obtained is the Human Y-chromosome TSPY Gene Positive Control (1E7 copies/μL).
2. Label six centrifuge microtubes as 6, 5, 4, 3, 2, and 1 respectively.
3. Add 45 μL of the Template Dilution Buffer into each tube. Use filtered pipette tips (the same applies hereinafter).
4. Transfer 5 μL of the Human Y-chromosome TSPY Gene Positive Control (1E7 copies/μL) into tube No. 6, and vortex vigorously for 1 minute. The concentration of the positive control in tube No. 6 is 1E6 copies/μL. Place the tube on ice for later use.
5. Change the pipette tip, transfer 5 μL of the diluent in tube No.6 into tube No. 5, and vortex vigorously for 1 minute. The concentration of the positive control in tube No. 5 is 1E5 copies/μL. Place the tube on ice for later use.
6. Change the pipette tip, transfer 5 μL of the diluent in tube No.5 into tube No. 4, and vortex vigorously for 1 minute. The concentration of the positive control in tube No. 4 is 1E4 copies/μL. Place the tube on ice for later use.
7. Repeat the above operation until six SDSC are obtained. Place them on ice for later use. They will be used as the templates for making the standard curve.

Section 2: Prepare samples and reagents

8. DNA purification: Perform N + 2 DNA purification for N specimens per the guide from the manufacture of the DNA purification kit. For the two extra purifications, one is for Purification Positive Control (PPC) using a known positive specimen as the starting material and the another for Purification Negative Control (PNC) using a known negative specimen as the starting material. Store the purified DNA at -20℃ until use.
9. Make Primer-Probe Mixture: When using the kit for the first time, add 220 μL Ultrapure Water into the microtube

that contains the Human Y-chromosome TSPY Gene and IC Primer-Probe Mix Lyophilized Powder. Vortex the microtube for 10 seconds and spin it down for 10 seconds. The solution thus obtained is Human Y-chromosome TSPY Gene and IC Primer-Probe Mixture. Put it on ice for later use. The leftover should be stored at -20°C.

10. Calculate the total number of PCR reactions: For N samples, if quantitative analysis is to be performed and only one repeat is to be done for each sample, the recommended reaction number is (N + 2) × 1 + 7 = N + 9. Among them, N + 2 reactions are for the N + 2 samples obtained in step 8, one is for the Amplification Negative Control (ANC, use water as the template), and six are for the six SDSC. Alternatively, if qualitative analysis is to be performed and only one repeat is to be done, the recommended reaction number is (N + 2) × 1 + 2 = N + 4. Among them, N + 2 reactions are for the N + 2 samples obtained in step 8, one is for the Amplification Negative Control (ANC use water as the template), and another for the Amplification Positive Control (APC, use the dilution obtained in step 6 as the template). If more than one repeat is tested for each sample, the total reaction numbers should be recalculated accordingly.

Section 3: PCR reaction set-up

11. Setup PCR reaction for quantitative analysis with one repeat for each sample: Assign wells of the 8-well strips to the N + 9 samples. Add each component into the N + 9 wells according to the following table. The six SDSC should be added after all other samples are added and the wells are capped:

Component	(N + 2) wells	ANC well	SDSC wells No. 1 – 6
Primer-Probe Mixture from step 9	4 μL each	4 μL	4 μL each
N + 2 DNA Samples from step 8	1 - 21 μL each	-	-
Six SDSC from step 7	-	-	1 - 21 μL each respectively
Ultrapure Water	To 25 μL each	To 25 μL	To 25 μL each

12. Alternatively, setup PCR reaction for qualitative analysis with one repeat for each sample: Add each component into the N + 4 labeled tubes according to the following table. The positive control should be added after all other samples are added and the wells are capped:

Component	(N + 2) tubes	ANC tube	APC tube
Primer-Probe Mixture from step 9	4 μL each	4 μL	4 μL
N + 2 DNA Samples from step 8	1 - 21 μL each	-	-
No. 4 dilution from step 6	-		1 - 21 μL each
Ultrapure Water	to 25 μL each	To 25 μL	To 25 μL

Section 4: Run PCR

13. Perform PCR with the parameter described in the following table:

Process	Temperature	Duration	Cycle Number
Denature	95°C	10 minutes	1 cycle
PCR	95°C	15 seconds	45 cycles
	52°C	15 seconds	
	72°C	60 seconds, collect signal in FAM channel and in Cy5 channel. Both quencher are BHQ3	

Note: For some models of PCR machines (e.g., Bio-Rad CFX96), if the three-step protocol does not yield satisfactory results, a two-step protocol can be used: 95°C for 10 minutes, followed by 45 cycles of (95°C for 15 seconds, 60°C for 60 seconds). Fluorescence signals are collected at the second step of each cycle. The annealing temperature may need to be optimized.

Section 5: Data Analysis and Interpretation

14. Determine the positivity or negativity of the results of all control reactions based on the following table:

Channel	Criteria	Positivity
FAM	Ct is smaller than 40 and the amplification plot is an inverted S-shaped curve	FAM Positive
	Ct is larger than 40	FAM Negative
	No Ct	FAM Negative
Cy5	Ct is smaller than 40 and the amplification plot is an inverted S-shaped curve	Cy5 Positive
	Ct is larger than 40	Cy5 Negative
	No Ct	Cy5 Negative

15. Validation of the purification experiment: If the PPC reaction is FAM positive and the PNC reaction FAM negative, the DNA purification process is valid. Move on to next step. If the PPC reaction is FAM negative or the PNC positive, the whole DNA purification process is invalid. There is no need to further analyze the data. The DNA purification process must be repeated. The possible reasons for invalid purification include the disfunction of the DNA purification kit, mistakes in experimental processes, failures of PCR machine, or carry-over contamination of the PNC samples from previous experiments or by other positive samples.
16. Validation of the amplification experiment: If No.2 - No.6 SDSC reactions are FAM positive and the ANC negative, the PCR amplification process is valid. Move on to next step. If any of the No.2 - No.6 SDSC reactions is FAM negative or the ANC positive, the whole PCR amplification experiment is invalid. There is no need to further analyze the data. The reason for invalid amplifications must be found. The possible reasons include the disfunction of the PCR reagents, the failure of PCR instruments, carry-over contamination of the ANC samples from previous experiments or by other positive samples.
17. If both purification and amplification experiments are valid, then analyze the results of the samples. For quantitative analysis, take the log value of the concentration of the six SDSC as the horizontal axis and the valid FAM Ct value as the vertical axis to draw a standard curve and to obtain its regression equation. Then, use the valid FAM Ct value of each sample to calculate from regression equation the log value of the DNA concentrations, which is converted to DNA concentration in copies/μL finally. If the FAM signal of one reaction is not detected or its FAM Ct is over 40, the positivity of the corresponding sample is dependent on the positivity of its IC. If the IC is positive, the FAM result is valid, which means the FAM reaction is negative. If its IC is negative, the FAM result is invalid, which means the purification and amplification process of this particular sample must be repeated.
18. For qualitative analysis, if the FAM signal is S-shaped and the Ct is below 40, the reaction of this sample is positive, no matter the Cy5 signal of the same reaction is positive or negative. If the FAM signal is not detected or the FAM Ct is over 40, the reaction of this sample is negative only when its Cy5 signal is positive. If both the FAM signal and the Cy5 signal is negative, this reaction is invalid and must be repeated.

Limited Product Warranty

- Skygen, Inc. warrants its products as set forth in the Skygen's General Terms and Conditions of Sale at www.skygen.bio. If you have any questions, please contact us order@skygen.bio.
- The information in this guide is subject to change without notice.
- This product is intended for research purposes only. This product is not intended to be used for therapeutic and diagnostic purposes in humans or animals.
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