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Catalog No: 15-32312

[Lactococcus lactis cremoris Probe Realtime PCR Kit]

User Manual

Disclaimer: Products are intended for research use only

skygen

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Product Description

Lactococcus lactis cremoris Probe Realtime PCR Kit is designed for the quantitative detection of Lactococcus lactis cremoris specific DNA in a real-time PCR test using hydrolysis probes. In the amplification process, Taq DNA polymerase amplifies the DNA sample via quantitative PCR using a primer-probe set that targets a Lactococcus lactis cremoris specific DNA region. At each cycle of the PCR, the 5′ nuclease activity of Taq DNA polymerase degrades the bound probes, releasing the quenched Lactococcus lactis cremoris-specific FAM signal. The increase in FAM fluorescence measures the Lactococcus lactis cremoris-specific DNA amplifications. At a point where the fluorescence signal is confidently detected over the background signal, a Ct value can be determined for the FAM signal. This Ct parameter can be used to evaluate relative target abundance among samples. Lactococcus lactis cremoris Probe Realtime PCR Kit has the following features:

1. One-tube PCR, minimized carry-over contamination.
2. High sensitivity, with an analytical LOD at 100 copies/reaction.
3. High specificity, no cross-reaction with DNA from other pathogens.
4. Amplification positive control is provided in the kit.
5. Compatible with various DNA purification methods.
6. Suitable for both qualitative and quantitative detection. For quantitative use, the linear range is at least 5 orders of magnitude.
7. Enough for 50 reactions, 20 μL per reaction.
8. Research use only.

Input Sample Requirements

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

Kit Components

Component Name	Product Number.	Size	Vial	Lid Color
2×Probe qPCR MasterMix	981201	0.5 mL	0.5 mL Vial	Natural
Template Dilution Buffer	180701	1 mL	1.5 mL Vial	Green
Lactococcus lactis cremoris Primer-Probe Powder	Yp15-32312	50 T	0.5 mL Brown Vial	Brown
Lactococcus lactis cremoris Amplification Positive Control (1E7 copies/μL)	pc32312	50 μL	0.5 mL Vial	Yellow
Ultrapure water	210806	1 mL	1.5 mL Vial	Blue
Manual	sc15-32312	1 Set	NA	NA

Storage and Handling

Reagents are shipped on ice and should be stored at -20℃ upon receipt. The kit is stable for at least one year from date of receipt. Repeated thawing and freezing of the kit components should be avoided. All components must be thawed at room temperature before use, placed on ice during use and stored at -20℃ after use. Thawed components should be mixed well by gently inverting or gently vortexing 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
Kit-specific laboratory equipment and consumables	Manual or automated DNA purification kits & equipments, real-time fluorescent PCR instruments 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/mL serial dilutions from the Positive Control.

Note: Since the concentration of the Lactococcus lactis cremoris Positive Control is very high, the following dilution operations must be carried out in an independent area, and the samples or other components of this kit must not be contaminated at all. The Positive Control is a DNA fragment from Lactococcus lactis cremoris genome and is not infectious.

1. Label six centrifuge microtubes as 6, 5, 4, 3, 2, and 1 respectively.
2. Add 45 μL of the Template Dilution Buffer into each tube. Use filtered pipette tips (the same applies hereinafter).
3. Transfer 5 mL of the Positive Control (provided in the kit, 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.
4. 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.
5. 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.
6. Repeat the above operation until six serial dilutions 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

7. 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 purification, One is for Purification Positive Control (PPC) using a known positive specimen as the starting material and the other is for Purification Negative Control (PNC) using a known negative specimen as the starting material. Store the purified DNA at -20℃ until use.
8. Make Prime-Probe Mix: When using the kit for the first time, add 165 μL Ultrapure water into the microtube that contains the Lactococcus lactis cremoris Primer-Probe Powder. Vortex the microtube for 10 seconds and spin it for 10 seconds.

   The solution is Lactococcus lactis cremoris Primer-Probe Mix. Put in ice for later use. The leftover should be stored

9. 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 7, one is for the Amplification Negative Control (ANC, use water as the template), and six are for the six diluents obtained in step 6. 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 t step 7, 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 5 as the template). If more than one repeat is tested for each samples, the total reaction numbers should be recalculated accordingly.

Section 3: Set up PCR reaction

10. Make PCR premix: In a 1.5 mL microtube, add the two components according to the following table:

Component	For 1 reaction	For N+4 reactions	For N+9 reactions	For X reactions
2×Probe qPCR MasterMix	10 μL	10 μL×(N+5)	10 μL×(N+10)	10 μL×(X+1)
Lactococcus lactis cremoris Primer-Probe Mix	3 μL	3 μL×(N+5)	3 μL×(N+10)	3 μL×(X+1)
Total	13 μL	13 μL×(N+5)	13 μL×(N+10)	13 μL×(X+1)

11. Setup PCR reaction for quantitative analysis with one repeat for each sample: Add each component into the N+9 labeled tubes according to the following table. The positive control or the six diluents for standard curve should be added after all other samples are added and the tubes are capped:

Component	(N+2) Tubes	ANC Tube	Standard Curve Tubes No. 1 – 6
PCR premix from step 10	13 μL each	13 μL	13 μL each
N+2 DNA Samples	7 μL each	-	-
Ultrapure water	-	7 μL	-
Six diluents from section 1	-	-	7 μL each respectively

12. 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 or the six diluents for standard curve should be added after all other samples are added and the tubes are capped:

Component	(N+2) Tubes	ANC Tube	APC Tube
PCR premix from step 10	13 μL each	13 μL	13 μL
N+2 DNA Samples	7 μL each	-	-
Ultrapure water	-	7 μL	-
1E4 copies/mL Dilution from step 5	-	-	7 μL

Section 4: Run PCR

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

Process	Temperature	Duration	Cycle Number
Denature	95℃	5 minutes	1 cycle
PCR	95℃	15 seconds	45 cycles
	60℃	30 seconds	
	72℃	1 minute, collect signal in FAM channel	

Section 5: Data Analysis and Interpretation

14. Validation of the test: After the PCR is complete, inspect the amplification plot to ensure that the baseline threshold was set within the PCR exponential phase and above any background signal. For basic information about data analysis on specific real-time PCR instruments, please refer to the user manual of the respective instrument.
15. Validation of the purification experiment: If the PPC result is positive and the PNC result is negative, the DNA Purification process is valid. Move on to next step. If the PPC reaction is negative or the PNC result is negative, the whole DNA Purification process is invalid. There is no need to further analyze the data of the sample. The DNA Purificaiton process must be repeated. The possible reasons of experimental failure include the disfunction of the DNA purification kit, mistakes in experimental processes, failures of PCR machine, or carry-over contamination of the environment from previous experiments.

16. Validation of the amplification experiment: If the APC result is positive and the ANC result is negative, the PCR amplification process is valid. Move on to next step. If the APC result is negative or the ANC result is positive, the whole PCR amplification experiment is invalid. There is no need to further analyze the results of the samples. The reason for PCR failures must be found. The possible reasons include the disfunction of the PCR amplification reagents and the failure of PCR instruments, or the carry-over contamination of the environment or of the reagents.

17. If both purification and amplification experiment are valid, then analyze the results of the samples. For quantitative analysis, take the log value of the concentration of the six standard curve dilutions as the horizontal axis and the valid Ct value as the vertical axis to draw a standard curve. Then, use the valid Ct value of the samples to be tested to calculate the log value of the DNA concentration from the standard curve, and then convert it to the DNA concentration in copies/mL.
18. For qualitative analysis, if there is no Ct value or the Ct value is greater than or equal to 40, the sample is negative. If the Ct value is less than 40, the sample is positive.

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