Cat. #CY228

For Research Use

TaKaRa

CycleavePCR™ *Staphylococcus aureus* (DnaJ gene) Detection Kit

Product Manual

v201904Da

Table of Contents

I. Description

Staphylococcus aureus is indigenous to human and animal skin and digestive tract. When it proliferates in food, *S. aureus* produces a toxin called enterotoxin, which causes food poisoning. This kit targets the DnaJ gene, a housekeeping gene that shows sequence diversity between bacterial species. This kit can specifically detect *S. aureus* when used in conjunction with a real-time PCR method. This kit includes *TaKaRa Ex Taq*® HS, a hot start PCR enzyme, which prevents non-specific amplifications caused by mispriming or primer dimer formation during reaction mixture preparation or other pre-cycling steps. The use of *TaKaRa Ex Taq* HS therefore allows high-sensitivity detections.

Amplification products are detected by cycling probe technology, which provides highly sensitive detections through the combined use of a RNA/DNA chimeric probe and RNase H. This enables efficient detection of specific sequences of the gene fragment during and after amplifications. One end of the probe is labeled with a fluorescent moiety and the other end with a quencher. When intact, this probe does not emit fluorescence due to the action of the quencher. However, when it forms a hybrid with the complementary sequence of an amplification product, RNase H cleaves RNA in the chimeric probe, resulting in strong fluorescent signal emission (see Figure 1). The amount of amplified product can be monitored by measuring the intensity of emitted fluorescence.

This kit includes an FAM-labeled probe for detecting amplification products from the DnaJ gene of *S. aureus* and a ROX-labeled probe for detecting amplification products from the internal control.

By simultaneously monitoring two wavelengths, DnaJ gene detection and false negative monitoring through detection of the internal control DNA can be achieved in a single tube. The kit relies on real-time PCR detection, which requires no electrophoresis and yields results quickly.

＊ This product was developed in collaboration with Dr. Takayuki Ezaki, professor of pathogen control, Gifu University School of Medicine.

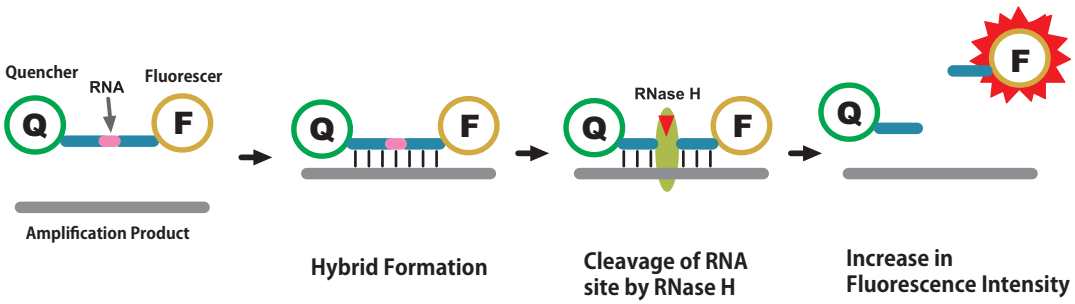


Figure 1. Principle of cycling probe technology

II. Components (for 50 reactions of 25 μl volume)

1.	2X Cycleave Reaction Mixture	625 μl
2.	*S. aureus* Primer/Probe Mix (FAM-, ROX-labeled)＊ (5X conc.)	250 μl
3.	dH_2O	1 ml
4.	*S. aureus* Positive Control	50 μl (10 reactions)

＊ Store protected from light, since it contains fluorescent-labeled probes.

[Component Information]

2X Cycleave Reaction Mixture:

PCR reaction reagents including enzymes, buffer, and dNTP mixture.

S. aureus Primer/Probe Mix:

This is a mixture of primers/probes for detecting the DnaJ gene of *S. aureus* and an internal control DNA. The DnaJ gene of *S. aureus* (the target gene) or the internal control DNA is amplified with the primers. The FAM-labeled probe detects DnaJ gene, and the ROX-labeled probe detects the internal control DNA.

Target gene:
The target gene is the DnaJ gene of *S. aureus*.

Internal control DNA:
The internal control DNA has no sequences related to the target gene. It works to detect false negatives. It is present in every reaction system supplied in this kit. When no target is detected, a positive detection of the internal control DNA indicates the absence of PCR inhibition and indicates that the concentration of target in the sample is below the detection limit. Absence of both target and internal control DNA detection indicates that PCR did not occur properly. When there is a large amount of the target DNA, the amplification of the target is prioritized, resulting in the delay, weakening, or absence of the internal control DNA signal. In such situations, the assay can be interpreted as positive.

dH_2O:
Sterile purified water

S. aureus Positive Control:
Positive control for the DnaJ gene of *S. aureus*

III. Storage

-20℃

IV. Materials Required but not Provided

[Instrument]

- Real-time PCR amplification instrument and tubes
  Thermal Cycler Dice Real™ Time System *II* (Cat. #TP900/TP960)＊1,2
  Thermal Cycler Dice Real Time System *Lite* (Cat. #TP700/TP760)＊1,2
  Applied Biosystems 7500 Fast Real-Time PCR System (Thermo Fisher Scientific), etc.
- Heat block (capable of temperature settings up to 95℃)

＊1 0.2-ml 8-strip tubes (with individual flat caps) for the Thermal Cycler Dice Real Time System are available for purchase. The use of such tubes is highly recommended for minimizing the risk of inter-tube contamination.
0.2 ml 8-strip tube, individual flat caps (Cat. #NJ600)＊2

＊2 Not available in all geographic locations. Check for availability in your area.

[Other]

- 1,000 μl, 200 μl, 20 μl, and 10 μl micropipettes
- Micropipette tips (with hydrophobic filter)
- Tabletop centrifuge
- Refrigerated microcentrifuge (with 4℃ setting)

V. Considerations Before Use

- This kit is designed to detect Staphylococcus DNA and can also detect non-viable bacteria. Staphylococcus DNA cannot be detected in some cases, when a mutation or deletion/insertion occurs within the sequence covered by the Primer/Probe Mix. (Takara Bio is not responsible for any actions taken as a result of analytical determinations made with this product.)
- When a sample is judged as positive, it should be verified also by microbioassay.

VI. Precautions

1) Operate real-time PCR instruments in accordance with the manufacturer's instructions.
2) The chimeric probe and primers are susceptible to degradation by nucleases, and if degraded, cannot provide accurate detection. Take care to avoid nuclease contamination from sources such as perspiration or saliva introduced during sample handling.
3) It is recommended to designate and physically segregate the 3 areas described below for the processes from preparation of reaction mixtures to detection. Avoid opening/closing tubes containing amplification products in any of these areas:
   ○ Area 1: reaction mixture preparation and dispensing
   ○ Area 2: sample preparation
   ○ Area 3: addition of samples to reaction mixtures, reaction, and detection

   This kit allows amplification and detection to take place simultaneously in real time. Thus, no electrophoresis or other analytical methods are required after the reaction is complete. Never remove amplification products from tubes, as doing so may introduce contamination.
4) Results obtained with this kit are interpreted based on analyses by a real-time PCR amplification instrument.
   Failure of any of the auto functions on the real-time PCR amplification instrument may lead to erroneous interpretation of results. Properly adjust the settings on the real-time PCR amplification instrument in accordance with the instrument manual when necessary.

VII. Protocol

[Protocol Overview]

1. Sample preparation
   Prepare bacterial heat-extracted samples from bacterial cultures.
2. Real-time PCR amplification instrument setup
3. Reaction mixture preparation and reaction start
   Prepare the reaction mixture
   ↓
   Dispense the reaction mixture into reaction tubes and add the negative control, the sample, or the positive control.
   ↓
   Set the tubes in the real-time PCR amplification instrument, then start the reaction.
   ↓
4. Displaying results
   The amplification curves are displayed.
   ↓
   Reaction completion
   ↓
   Interpretation

VII-1. Sample preparation (work in Area 2)

[Preparation of heat-extracted bacterial samples]

(1) Pipet 10 μl of the bacterial culture into a 1.5-ml tube.
(2) Add 90 μl of sterile water and mix.
(3) Incubate at 95℃ for 5 min.
(4) Centrifuge at 12,000 rpm, 4℃ for 10 min, and then collect the supernatant for use as the heat-extracted sample for DnaJ gene detection. Use 5 μl per reaction.

- ＊ If the PCR reaction was inhibited when using the heat-extracted samples prepared by this method, try the PCR reaction again using 10-fold and 100-fold diluted samples. Use purified water to prepare the dilutions.
- ＊ Prepare bacterial cultures from food samples in accordance with the applicable standard protocol. Heat-extracted samples may be stored at -20℃.

VII-2. Reaction mixture preparation and starting the reaction

This kit allows the simultaneous detection of amplification products from both the DnaJ gene and the internal control in a single reaction tube. To obtain accurate detection results, perform the positive control reaction and the negative control reaction for the DnaJ gene simultaneously.

(1) Prepare the following reaction mixture on ice. (Work in Area 1)

Prepare components other than the sample template in volumes sufficient for the required number of tubes plus a few extra. Dispense aliquots of 20 μl into PCR tubes and cap loosely. Use one of the tubes as a negative control by adding 5 μl of dH_2O and then cap the tube tightly.

The required number of tubes is defined as the number of samples + 2 (one for the negative control reaction and one for the positive control reaction).

Reagent	Volume (per reaction)	Final Conc.
2X Cycleave Reaction Mixture	12.5 μl	1X
S. aureus Primer/Probe Mix (5X conc.)	5 μl	1X
Sample or Positive Control or dH_2O	(5 μl)*	
dH_2O	2.5 μl	
Total	25 μl	

* Add the sample or the positive control DNA in step (2), not in this step.

[Precaution]
Do not touch tubes and caps with bare hands; doing so may introduce noise during detection of fluorescent signal.

(2) Add sample (template). (Work in Area 3)

Add either the sample or the positive control (*S. aureus* Positive Control) to each tube except the negative control tube and then cap tightly.

Briefly centrifuge the tubes in a table top centrifuge and then set them in a real-time PCR instrument.

[Precaution]
Start reactions within 1 hour of preparing the reaction mixtures.

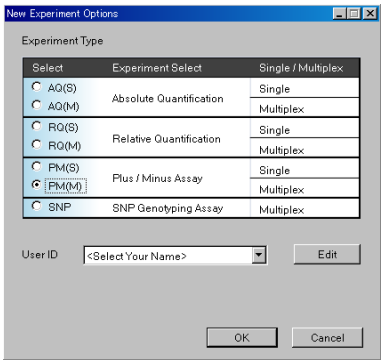
VII-3. Amplification and detection by real-time PCR and interpretation of results (Work in Area 3)

Operating procedures differ depending on the real-time PCR instrument used. For specific operating procedures, see the instrument manual.

An overview of operation and analysis is described here for the Thermal Cycler Dice Real Time System *III* and the Applied Biosystems 7500 Fast Real-Time PCR System (Thermo Fisher Scientific).

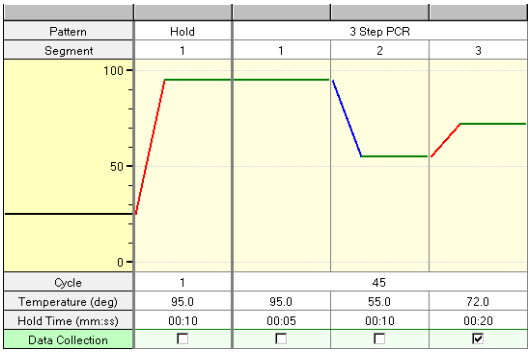
[Thermal Cycler Dice Real Time System *III*]

(1) Open a new run file. On the "New Experiment Options" screen, select analysis type: "Plus/Minus Assay Multiplex"



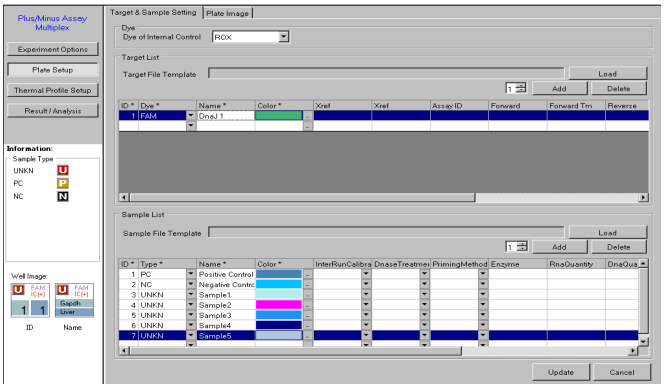
(2) On the "Thermal Profile Setup" screen, make sure both detection filters, FAM and ROX, are checked and the PCR conditions are set as follows:

Initial denaturation (Hold)
Cycle: 1
95℃ 10 sec
3 step PCR
Cycle: 45
95℃ 5 sec
55℃ 10 sec
72℃ 20 sec (detection)



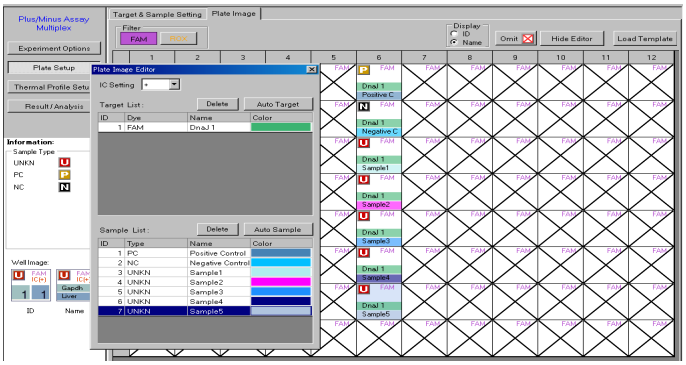
(3) Click the "Start Run" button on the bottom right-hand corner of the screen and enter a run file name to start the reactions.

(4) On the “Plate Setup” screen, enter the sample information.
For the internal control "Dye" setting, select "ROX". After targets and sample information are entered, click the "Update" button.



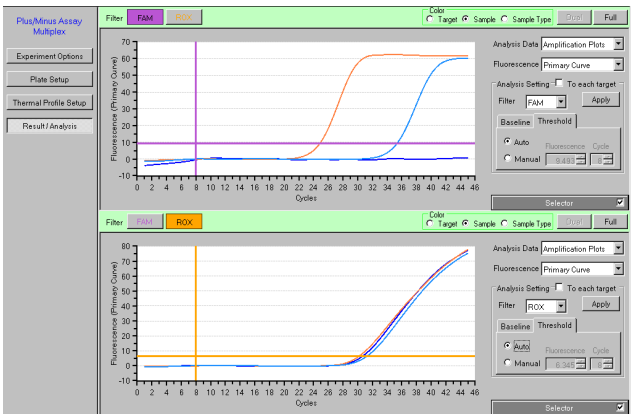
＊ To maximize the reliability of result interpretation, it is recommended to run at least 2 strips of reactions.

After clicking the "Plate Image" tab, select the desired wells. Select "Omit" for unused wells.



(5) Analysis of results

1. After reactions are complete, click the “Result/Analysis” button.

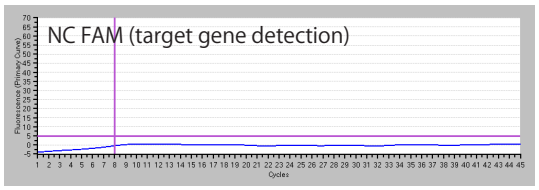


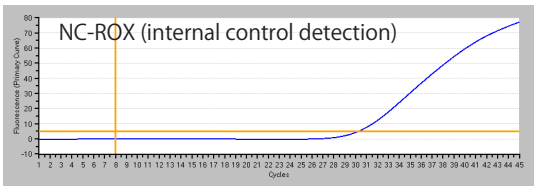
Two screens are displayed: the amplification curve of the target-detecting FAM filter at the top and the amplification curve of the internal control-detecting ROX filter at the bottom. (The threshold setting is “Auto”.)

2. Check amplification curves for NC (negative control) and PC (positive control). Make sure the baseline provided by the FAM filter for NC is free of fluorescent signal changes and is below the threshold.

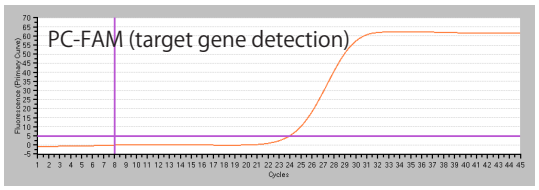
   If the threshold is exceeded, manually enter the threshold setting so that the baseline stays below the threshold.

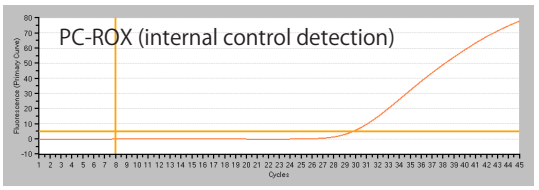
   Make sure the amplification curve for the ROX filter is displayed and that the curve exceeds the threshold.



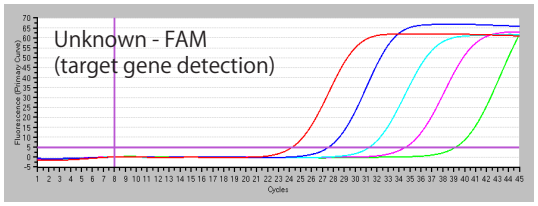


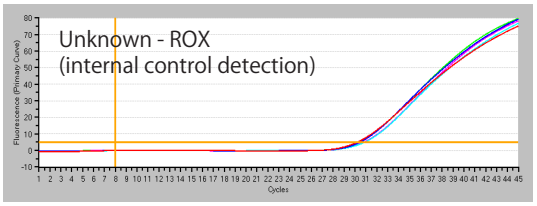
   Make sure the amplification curve for PC's FAM filter is displayed and exceeds the threshold and that the amplification curve for the ROX filter is displayed and exceeds the threshold.



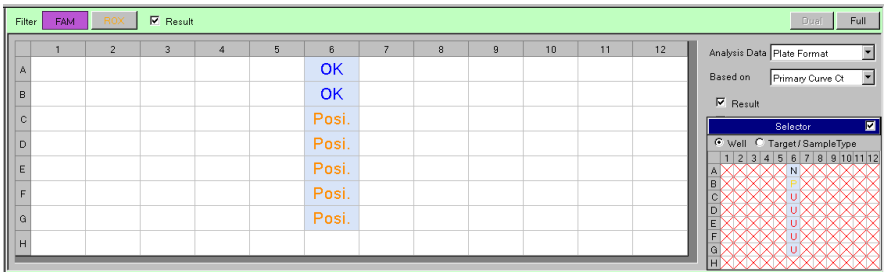


3. On the "Selector", select "U" to display the results. Then make sure the baseline and the amplification curve for the FAM filter are displayed with no abnormality.





4. On the "Analysis Data" column, display "Plate Format"



OK : The control reaction is normal (indicating the reaction performed correctly).
OUT : The control reaction is abnormal (indicating the reaction did not perform correctly).
Posi : The target gene detection is positive.
Nega : The target gene is below the limit of detection.
ND : Neither the internal control nor the target gene is detected; no interpretation available (indicating the PCR reaction failed to occur properly)
Error : Indicating different result interpretations within the same replicate.

■ Precautions for result interpretation

- When the FAM filter (target gene detection) shows an amplification curve (the result interpretation is "OUT") in the negative control reaction (NC).
  → Contamination may have occurred. Decontaminate the bench area used to prepare reaction mixtures as well as the apparatuses and instruments used. Then perform the reaction again.
- When both the FAM filter and the ROX filter (internal control detection) show no amplification curve (the result interpretation is "OUT") in the positive control reaction (PC).
  → The PCR reaction or the cycling probe detection failed to work properly. Repeat the reaction.
- When the ROX filter shows an amplification curve but the FAM filter does not show an amplification curve (the result interpretation is "OUT") in the positive control reaction (PC).
  → There may be a problem with the Primer/Probe Mix or the positive control may have been degraded.
- When both the FAM filter and the ROX filter do not show an amplification curve (the result interpretation is "ND") in the sample reaction (UNKN).
  → The PCR or the cycling probe detection failed to work properly. Repeat the reaction.
  The sample may contain a reaction inhibitor. Perform the reaction again with diluted samples. Alternatively, prepare the sample again and then perform the reaction again.
- When, in the sample reaction (UNKN), the FAM filter yielded an amplification curve but the ROX filter did not.
  → When there is a large amount of target DNA, the amplification of the target may be prioritized, resulting in the competitive inhibition of the amplification reaction for the internal control DNA. (The result interpretation displayed is "Posi") The detection system performed properly.

[For the Applied Biosystems 7500 Fast Real-Time PCR System, StepOnePlus™ Real-Time PCR System (Thermo Fisher Scientific)]

Analyzed in Quantification-Standard Curve mode; to adjust various settings, go to Advanced Setup

PCR Conditions
Initial Denaturation（Hold）
Cycle：1
95℃ 10 sec
3 step PCR
Cycles：45
95℃ 5 sec
55℃ 10 sec
72℃ 20 sec (detection）

Passive Reference
(none)

Define Targets
Target Name : DnaJ, Reporter : FAM, Quencher : (none)
Target Name : IC, Reporter : ROX, Quencher : (none)

Define Samples
Negative Control
Sample Type : NTC (No Template Control)
Test Samples, Positive Control
Sample Type : Unknown

Note : With the StepOnePlus Real-time PCR System (Thermo Fisher Scientific), the detection sensitivity for ROX is low, and the ROX (IC) amplification curve will appear small when all targets are displayed simultaneously. Analyze the FAM and ROX targets displayed separately.

VIII. Interpretation

For samples reaction :

Note: Perform a final interpretation of the assay based on all results including those of the control reactions.

	Amplification Signal	ROX (internal control)	
		(+)	(−)
FAM (DnaJ)	(+)	DnaJ, positive＊1	DnaJ, positive＊1
	(−)	DnaJ, below the limit of detection＊2	No data available＊3

Positive Control reaction (using the *S. aureus* Positive Control)

	Amplification Signal	ROX (internal control)	
		(+)	(−)
FAM (DnaJ)	(+)	No problem with the reaction	No problem with the reaction
	(−)	Problem with the reaction＊4	No data available＊3

Negative Control reaction (using sterile purified water)

	Amplification Signal	ROX (internal control)	
		(+)	(−)
FAM (DnaJ)	(+)	Possible contamination in the reaction＊5	Possible contamination in the reaction＊5
	(−)	No contamination in the reaction	No data available＊3

＊1 Whether the results for detection of the internal control DNA are positive or negative, the result for the DnaJ gene is positive. Confirm that there is no contamination in the reaction system with the results of the negative control reaction.

＊2 Verify that the result is (+) for the Positive Control detection (indicating no problem with the reaction).

＊3 The PCR reaction or the cycling probe detection failed to work properly for some reason. Perform the reaction again. Because the sample may contain a reaction inhibitor, it may be necessary to prepare the sample again.

＊4 There is either problem with the *S. aureus* Primer/Probe Mix, or the *S. aureus* Positive Control is degraded.

＊5 Decontaminate the bench area and apparatuses used for preparing reaction mixtures.

IX. Validation

Validation of detectable bacterial strains: ○ : Detectable × : Not detected

No.	GTC No.	Genus	Species epithet	Type strain	Detection
1	1770	*Staphylococcus*	*aureus* enterotoxin A producer		○
2	1779	*Staphylococcus*	*aureus* enterotoxin B producer		○
3	1772	*Staphylococcus*	*aureus* enterotoxin B&C producer		○
4	1773	*Staphylococcus*	*aureus* enterotoxin D producer		○
5	286	*Staphylococcus*	*aureus*	Type strain	○
6	12388	*Staphylococcus*	*aureus* MRSA		○
7	371	*Staphylococcus*	*aureus* subsp. *anaerobius*	Type strain	○
8	9120	*Staphylococcus*	*aureus* subsp. *aureus*	Type strain	○
9	727	*Staphylococcus*	*capitis* subsp. *reolyticus*	Type strain	×
10	287	*Staphylococcus*	*capitis* subsp. capitis	Type strain	×
11	378	*Staphylococcus*	*caprae*	Type strain	×
12	384	*Staphylococcus*	*carnosus*	Type strain	×
13	380	*Staphylococcus*	*caseolyticus*	Type strain	×
14	482	*Staphylococcus*	*chromogenes*	Type strain	×
15	248	*Staphylococcus*	*cohnii* subsp. *cohnii*	Type strain	×
16	289	*Staphylococcus*	*epidermidis*	Type strain	×
17	509	*Staphylococcus*	*felis*	Type strain	×
18	290	*Staphylococcus*	*haemolyticus*	Type strain	×
19	485	*Staphylococcus*	*hominis*	Type strain	×
20	305	*Staphylococcus*	*hyicus*	Type strain	×
21	266	*Staphylococcus*	*intermedius*	Type strain	×
22	382	*Staphylococcus*	*kloosii*	Type strain	×
23	458	*Staphylococcus*	*lugdnensis*	Type strain	×
24	705	*Staphylococcus*	*pasteuri*	Type strain	×
25	181	*Staphylococcus*	*saccharolyticus*	Type strain	×
26	265	*Staphylococcus*	*saprophyticus*	Type strain	×
27	11875	*Staphylococcus*	*shleiferi*	Type strain	×
28	292	*Staphylococcus*	*simulans*	Type strain	×
29	293	*Staphylococcus*	*warneri*	Type strain	×
30	294	*Staphylococcus*	*xylosus*	Type strain	×

GTC: Gifu University Type Culture Collection

X. Related Products

Thermal Cycler Dice™ Real Time System *III*（Cat. #TP900/TP960）*
Thermal Cycler Dice™ Real Time System *Lite*（Cat. #TP700/TP760）*
96 well Hi-Plate for Real Time（Cat. #NJ400）
Sealing Film for Real Time（Cat. #NJ500）
Plate Sealing Pads（Cat. #9090）
0.2 ml Hi-8-tube（Cat. #NJ300）
0.2 ml Hi-8-Flat Cap（Cat. #NJ302）
48 well snap plate (Cat. #NJ700)
Flat cap for snap plate (Cat. #NJ720)
0.2 ml 8-strip tube, individual Flat Caps（Cat. #NJ600）*

* Not available in all geographic locations. Check for availability in your area.