Cat. # RR240A

For Research Use

TaKaRa

Bacteria (*tuf* gene) Quantitative PCR Kit

Product Manual

v202506Da

Table of Contents

I. Description

The Bacteria (*tuf* gene) Quantitative PCR Kit is intended for the quantification of bacterial number using real-time PCR. This kit can generate results more quickly than conventional methods that require 2 - 4 days of culture, and can be used for bacterial detection in the food safety and environmental research fields. Furthermore, with conventional methods, differences in optimal culture conditions for different bacteria make it necessary to use specialized culture conditions and/or media to detect various types of bacteria present in the specimen [1 - 3]. This kit allows the total number of a wide variety of bacterial strains to be measured without any specialized culture conditions.

Target Genes

PCR detection of general bacterial strains using the 16S rRNA gene is possible. But this analysis is not suitable for precise quantification of bacterial number because the copy number of 16S rRNA varies among bacterial strains. The target gene of this kit is the protein elongation factor Tu (*tuf*) gene. The *tuf* gene has a high degree of conservation among various bacterial strains, and has a chromosomal copy number of one or two [4].

Real-Time PCR

Real-time PCR is a method of gene detection that monitors incorporation of fluorescent moieties in real time during the PCR amplification process. This intercalator method offers excellent speed and quantitative performance. The TB Green® *Premix Ex Taq*™ GC reagent that is included with this kit uses TB Green as an intercalator for detection and is capable of efficiently amplifying sequences with high GC content, making it suitable for detection of the *tuf* gene in a wide range of strains.

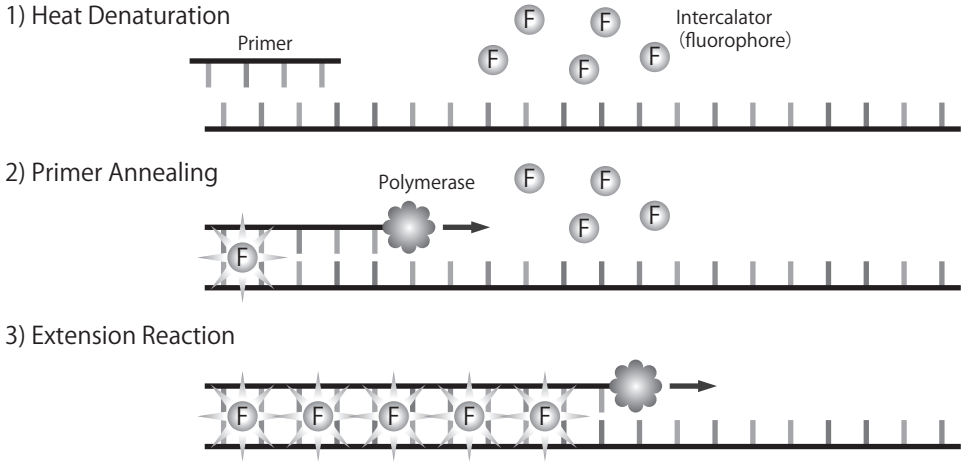


Figure 1. Fluorescent intercalator detection method.
The intercalator fluoresces when incorporated into dsDNA during PCR amplification.

Note: Drs. Bon Kimura and Hajime Takahashi of Tokyo University of Marine Science and Technology and Dr. Yuichiro Tanaka of TOYO SUISAN KAISHA, LTD. participated in the development of this kit.

II. Components (100 Reactions, 25 μl volume per reaction)

TB Green *Premix Ex Taq* GC*1	2X conc.	625 μl x 2
TUF Primer Mix	5X conc.	250 μl x 2
dH_2O		1 ml
ROX Reference Dye*2	50X conc.	50 μl
ROX Reference Dye II*2	50X conc.	50 μl
TUF Positive Control	1 x 10^5 copies/μl	100 μl
EASY Dilution (for Real Time PCR)		1 ml x 2

＊1 Includes *TaKaRa Ex Taq*® HS, dNTP Mixture, Mg^{2+}, and TB Green.

＊2 This component is to be used for analyses using a device that corrects fluorescent signals between wells, such as the real-time PCR devices by Applied Biosystems.

◆ Use the ROX Reference Dye
- Applied Biosystems StepOnePlus Real-Time PCR System (Thermo Fisher Scientific)

◆ Use the ROX Reference Dye II
- Applied Biosystems 7500 Fast Real-Time PCR System (Thermo Fisher Scientific)

◆ Do not use this component
- Thermal Cycler Dice™ Real Time System IV with PC (Cat. #TP1010)
- Thermal Cycler Dice Real Time System *II* (Cat. #TP900/TP960: discontinued)
- Thermal Cycler Dice Real Time System *Lite* (Cat. #TP700/TP760: discontinued)

III. Storage

TB Green *Premix Ex Taq* GC (Package 1)

4℃ (stable for 6 months)
-80℃ (for long-term storage)

Note: Avoid storage at -20℃. Once the product is thawed, store at 4℃ and use within 6 months.
Please be sure to shield from light during storage. Furthermore, be aware of the risk of contamination when storing at 4℃.

Other Components (Package 2)

Store at -20℃

IV. Materials Required but Not Provided

<Preparation of Sample Suspensions>
Dilution solution (0.1% peptone in physiological saline, etc. based on the specimen type)
Stomacher and Stomacher bag

<DNA Extraction>
NucleoSpin Tissue (Cat. #740952.10/.50/.250)*
Ethanol (> 99%)
Heat block (Set to 56℃ and 70℃)
Micropipette
Micropipette tips (with hydrophobic filters)
High-speed microcentrifuge
20 mg/ml lysozyme in 20 mM Tris-HCl, 2 mM EDTA, 1% Triton X-100 (pH 8.0)

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

<Real-Time PCR>
Amplification equipment for real-time PCR and tubes
Thermal Cycler Dice Real Time System IV with PC (Cat. #TP1010)
Thermal Cycler Dice Real Time System // (Cat. #TP900/TP960: discontinued)
Thermal Cycler Dice Real Time System *Lite* (Cat. #TP700/TP760: discontinued)
Applied Biosystems 7500 Fast Real-Time PCR System (Thermo Fisher Scientific)
Applied Biosystems StepOnePlus Real-Time PCR System (Thermo Fisher Scientific)
Benchtop Centrifuge

V. Precautions for Use

These are precautions for using this kit. Be sure to read before use.

1. Intended Use : This kit is a product for use in food and environmental testing. This is intended for research use only.

2. Results of Assay : This product is designed for detection of the target gene DNA, and detects not only living bacteria but also inactivated bacteria. The target gene may not be detected when there is mutation, deletion, or insertion in the genomic sequences correspond with the primers. (Takara Bio is not responsible for any actions taken as a result of analytical determinations made with this product.)

3. Disposal : Samples should be handled according to regulations governing use of potentially infectious materials. Dispose of materials according to the safety regulations for your facility and in accordance with any applicable local, state, or federal regulations. Keep the work area sanitized at all times and sterilize all samples and equipment used during the experiment. Sterilization may be conducted by autoclaving at 121℃ for at least 20 min or by treatment with 2.5% sodium hypochlorite, followed by processing materials according to guidelines for potentially infectious waste. Process and dispose of plastic and filter paper reagent containers and instruments according to regulations concerning the handling of hazardous materials.

VI. Precautions

1. TB Green *Premix Ex Taq* GC contains enzymes. Before use, make sure the reagent is evenly mixed by gently inverting several times without creating bubbles; if inadequately mixed, it may not provide sufficient reactivity. Do not mix by vortexing.

   Please note that a white to yellowish-white precipitate may form when TB Green *Premix Ex Taq* GC is stored at -80℃. This will dissolve completely when the solution is hand warmed gently or when it is left at room temperature for a short period (protect from light) and then mixed by inversion. Failure to redissolve the precipitate may result in unevenness of the reagent components; be sure to mix until homogenous before use.

2. Place reagents on ice when preparing the reaction solution.

3. TB Green *Premix Ex Taq* GC includes TB Green. Do not to expose to strong light during preparation of the reaction solution.

4. We recommend that the three following areas be established from preparation of reaction mixture to addition of samples in the laboratory working space and be physically separated from each other. 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

   This kit allows the amplification reaction and detection to take place in real time. Therefore, the amplification products require no electrophoresis or other treatment after the reaction is complete. Never remove amplification products from tubes, as doing so may cause nucleic acid contamination in the laboratory workspace.

VII. Protocol

Overview

Sample Preparation

Specimen (10 g) + Dilution Solution (90 ml)
↓
Stomacher Processing
↓

DNA Extraction

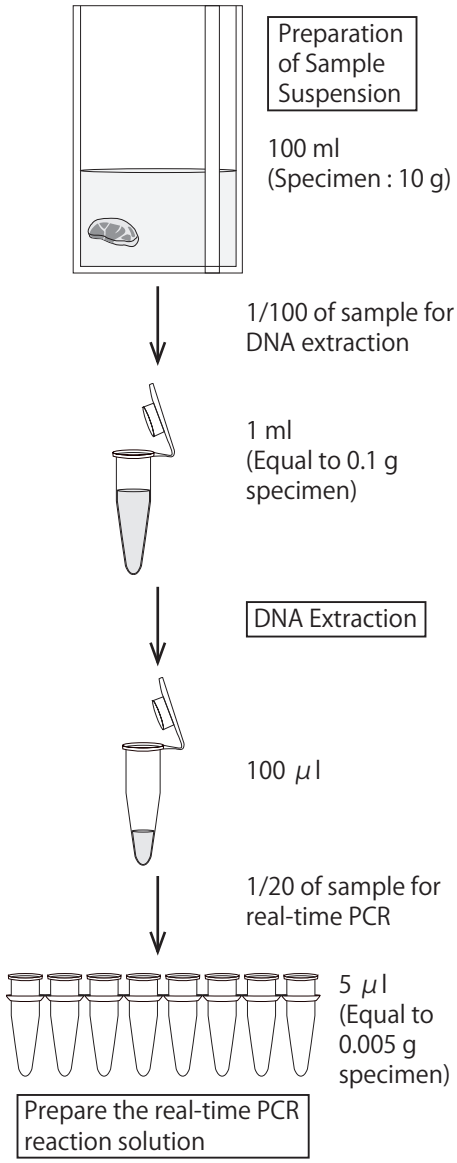
Centrifuge 1 ml of the specimen solution (equivalent to 0.1 mg of the specimen) at 8,000*g* for 5 min and remove the supernatant.
↓
DNA extraction from the precipitate using NucleoSpin Tissue (Elution volume: 100 µl)
↓

Preparation of Real-Time PCR Reaction Solution and Start of Reaction

Make serial dilutions of the TUF Positive Control and prepare standards for the standard curve.
↓
Prepare the reaction solution.
↓
Dispense the reaction solution into the reaction tubes and add the negative control (H_2O), standards, and 5 µl of the specimen sample (equivalent to 0.005 g of the specimen).
↓
Place the reaction tubes in the amplification device for real-time PCR and begin the reaction.
↓

Quantitative Analysis

Use the analysis software for the real-time PCR instrument to quantify *tuf* for the specimen sample based on the standard curve.



VII-1. Preparation of the Sample Suspension (Work in area 2)

1. Weigh 10 g of the specimen and transfer to a Stomacher bag, etc.
2. Add 90 ml of dilution solution (such as 0.1% peptone in physiological saline) (equivalent to 9 times the specimen).
3. Homogenize as necessary using a Stomacher, etc. and use as the specimen solution for DNA extraction.

VII-2. DNA Extraction (Work in area 2)

Perform DNA extraction according to the NucleoSpin Tissue protocol for bacteria (Gram-positive bacteria) and elute in 100 µl.

1. Dispense 1 ml of the specimen solution into a 1.5 ml microtube.
2. Centrifuge for 5 min at 8,000*g* and remove the supernatant.
3. Suspend the pellet in 180 µl of 20 mg/ml lysozyme in 20 mM Tris-HCl, 2 mM EDTA, 1% Triton X-100 (pH 8.0)*1 and incubate for 30 - 60 min at 37℃.
4. Add 25 µl of Proteinase K*2 and incubate at 56℃ for 1 - 3 hours (or overnight) until completely dissolved.
5. Agitate the sample. Add 200 µl of Lysis Buffer B3, agitate strongly, and then incubate for 10 min at 70℃. If undissolved material remains, centrifuge for 5 min at 11,000*g* and transfer the supernatant to a new tube.
6. Add 210 µl of ethanol (> 99%) to the supernatant and mix well.
7. Place the NucleoSpin Tissue Column in the Collection Tubes (2 mL). Transfer the above solution to the Column and centrifuge for 1 min at 11,000*g*. After discarding the filtrate, place the Column in the same Collection Tube.
8. 1st Wash : Add 500 µl of Wash Buffer BW to the Column and centrifuge for 1 min at 11,000*g*. After discarding the filtrate, place the Column in the same Collection Tube.

   2nd Wash : Add 600 µl of Wash Buffer B5*3 to the Column and centrifuge for 1 min at 11,000*g*. After discarding the filtrate, place the Column in the same Collection Tube.
9. Centrifuge the Column for 1 min at 11,000*g*.
10. Place the Column in a 1.5 ml microtube (provided by the user). Add 100 µl of Elution Buffer BE that has been heated to 70℃, incubate for 1 min at room temperature, and centrifuge for 1 min at 11,000*g*.

    Store the eluted DNA solution at 4℃ and use as the template sample for real-time PCR. For long-term storage, store at -20℃, and avoid repeated freezing and thawing.

＊1 Not included in NucleoSpin Tissue.

＊2 Preparation of Proteinase K solution
When using NucleoSpin Tissue Cat. #740952.10 :
Add 260 µl of Proteinase Buffer PB to 6 mg of Proteinase K (lyophilized) and dissolve completely. Store the prepared Proteinase K solution at -20℃ (stable for 6 months).

＊3 Preparation of Wash Buffer B5 solution
When using NucleoSpin Tissue Cat. #740952.10 :
Add 16 ml of ethanol (96 - 100%) to 4 ml of Wash Buffer B5 (Concentrate).

VII-3. Real-Time PCR

1. Preparation of the samples for the standard curve (Work in area 3)
   Make serial dilutions of the TUF Positive Control using EASY Dilution to obtain the samples for the standard curve.

   (1) 1 x 10^5 copies/ μl (TUF Positive Control stock solution)
   (2) 1 x 10^4 copies/ μl (5 μl TUF Positive Control stock solution + 45 μl EASY Dilution)
   (3) 1 x 10^3 copies/ μl (5 μl of 1 x 10^4 copies/ μl solution + 45 μl EASY Dilution)
   (4) 1 x 10^2 copies/ μl (5 μl of 1 x 10^3 copies/ μl solution + 45 μl EASY Dilution)

   Using these samples, it is possible to obtain quantitative values in terms of copy number of *tuf* gene per gram of specimen from the real-time PCR data (Refer to the section "VIII. Analysis" for the calculation methods).

   Table 1. Comparison of Copies/Reaction and Copies/g specimen

	copies/reaction (Copy Number per Reaction)	copies/g specimen (Copy Number per Gram of Specimen)
1	5 x 10^5	1 x 10^8
2	5 x 10^4	1 x 10^7
3	5 x 10^3	1 x 10^6
4	5 x 10^2	1 x 10^5

2. Preparation of the Reaction Solution (Work in area 1)
   Prepare the reaction mixture on ice.
   Prepare enough for the required number of tubes plus some extra, dispense 20 μl into each PCR reaction tube, and close the caps lightly. Add 5 μl of sterile purified water in place of the DNA sample to one tube as a negative control and close the cap tightly.

   [For the Thermal Cycler Dice Real Time System]

Reagents	(per reaction)
TB Green *Premix Ex Taq* GC (2X conc.)	12.5 μl
TUF Primer Mix (5X conc.)	5.0 μl
dH_2O	2.5 μl
DNA sample or standard curve sample	(5.0 μl)*2
Total	25.0 μl

   [For Applied Biosystems Real-Time PCR Instruments]

Reagents	(per reaction)
TB Green *Premix Ex Taq* GC (2X conc.)	12.5 μl
TUF Primer Mix (5X conc.)	5.0 μl
ROX Reference Dye or ROX Reference Dye II*1	0.5 μl
dH_2O	2.0 μl
DNA sample or standard curve sample	(5.0 μl)*2
Total	25.0 μl

   *1 ROX Reference Dye is used for the StepOnePlus, and ROX Reference Dye II is used for the 7500 Fast Real-Time PCR System.

   *2 The DNA sample and standard curve samples should be added in step 3, NOT in this step.

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

3. Addition of the Sample (DNA Solution) (Work in area 3)
   Add 5 μl of sample (specimen DNA sample, standard curve samples, etc.) to the PCR reaction solution and close the caps tightly. Centrifuge briefly in a benchtop centrifuge and place in the real-time PCR amplification instrument.

4. Real-Time PCR Reaction
   Perform the following PCR conditions.
   * Please refer to the instruction manual for the real-time PCR amplification instrument for specific operating procedures.
   * When analyzing data from Thermal Cycler Dice Real Time System, ensure that the normalization correction setting is turned OFF. For instructions on how to change the normalization correction setting, please refer to the user manual for the instrument.

◆ For the Thermal Cycler Dice Real Time System IV

Note: Please select "Fast" for the Speed setting on the Thermal Profile Setup screen.

Initial Denaturation
95℃ 30 sec
2 Step PCR
35 cycles
95℃ 5 sec
60℃ 1 min (Detection of Fluorescence: FAM)
Melt curve analysis

◆ For the other real-time PCR amplification instruments

Note: Please select "Fast" for the Run mode/Ramp speed.

Initial Denaturation
95℃ 30 sec
2 Step PCR
35 cycles
95℃ 5 sec
60℃ 30 sec (Detection of Fluorescence: FAM)
Melt curve analysis

[Caution] The sequence of PCR products amplified using samples and the TUF Primer Mix may differ from that of the TUF Positive Control. In dissociation analysis of real-time PCR, the Tm values of the PCR products differ between the sample and the TUF Positive Control. The copy number can also be correctly calculated under these conditions.

VIII. Analysis

VIII-1. Principles of Quantification

TUF Positive Control

The TUF Positive Control included in this kit is plasmid DNA that contains the *tuf* gene region and is adjusted to 1 x 10^5 copies/ μl (based on the OD_{260}). When serially diluted, it can be used to obtain a standard curve and quantify experimental samples.

Note : This kit detects both viable and non-viable bacteria. If you need to detect only viable bacteria, perform a culture test as well.

Calculation of the Copy Number per Gram of Specimen

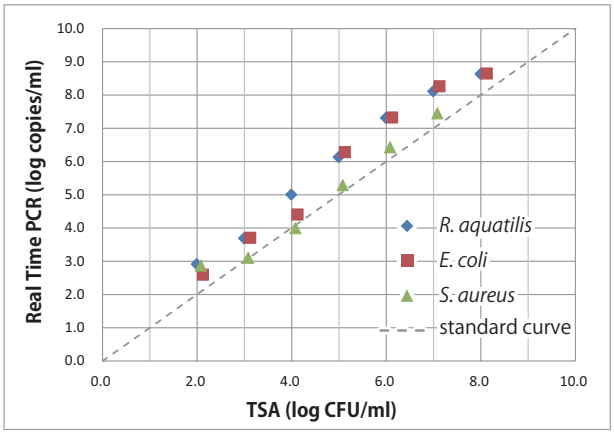
When this product is used according to the instructions, an amount equivalent to 0.005 g of the specimen is used for a real-time PCR reaction, and the value of tuf gene copies/reaction can be multiplied by 200 to obtain the copy number of *tuf* gene per gram specimen.

10 g of the specimen + 90 ml of dilution solution
↓ Stomacher treatment
1 ml of the specimen solution (equivalent to 0.1 g of the specimen)
↓ DNA extraction
100 μl of the DNA solution (equivalent to 0.1 g of the specimen)
↓ 1/20 to real-time PCR
5 μl per reaction used in real-time PCR (equivalent to 0.005 g of the specimen)

VIII-2. Relationship between the Gene Copy Number and Bacterial Cell Number

Cultured Bacteria

Rahnella aquatilis, Escherichia coli, and *Staphylococcus aureus* bacterial strains were cultured overnight in TSB media and each was serially diluted using physiological saline. Viable cell number was determined by culturing on TSA agar plates. Additionally, 1 ml of the bacterial suspensions were analyzed by real-time PCR analysis according to the instructions for this kit.



The cell number on TSA plates (colony forming units: CFU) are plotted on the X-axis, and the gene copy number obtained from real-time PCR are plotted on the Y-axis. There is good correlation between bacterial cell number and gene copy number. While there was a tendency for the copy number calculated to be somewhat high, the difference was limited to no more than one order of magnitude.

Figure 2. The relationship between *tuf* gene copy number and bacterial cell number.

Food Specimens

10% suspensions of 9 vegetables, 6 salads (including cut vegetables), and 8 meats were prepared. Measurement of cell number using TSA plate cultures (30℃, 48 hours) and real-time PCR analysis using this kit were carried out.

Table 2. Gene Copy number and cell number (food specimens)

	qPCR	TSA
	[log copies/g]	[log CFU/g]
Cabbage	1.51	0.90
Mizuna	3.38	3.20
Sprouts	4.61	3.30
Bok Choy	6.63	6.57
Cabbage	7.08	5.60
Lettuce	7.27	6.33
Mizuna	8.02	7.24
Sprouts	8.29	7.07
Mitsuba	8.65	7.10
Shredded Cabbage	2.66	2.48
Cut Lettuce	3.27	2.96
Cut Lettuce	5.09	4.41
Mixed Vegetable Salad	6.55	5.39
Green Salad	7.13	6.21
Mixed Salad	8.20	6.53
Beef	1.55	1.99
Pork	3.50	2.78
Sliced Pork	4.86	5.15
Ground Meat	5.47	4.89
Ground Chicken	5.53	4.17
Pork Loin	6.08	5.41
Chicken Thigh	6.20	4.97
Sliced Beef	6.28	6.25

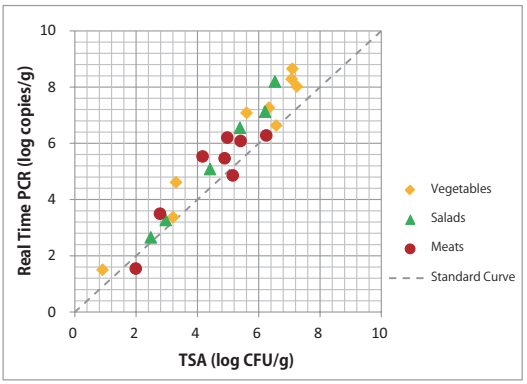


Figure 3. Relationship between *tuf* copies and cell number in food specimens.

The results from TSA cultures (CFU/g) are plotted on the X-axis, while the results of real-time PCR (copies/g) are plotted on the Y-axis. There is good correlation between the results from the cultures and real-time PCR. There was a tendency for the copy number to be 1 - 2 orders of magnitude higher than the bacterial cell number. This may be due to the presence of dead cells and/or bacterial strains that are difficult to detect by culturing.

IX. Appendix 1

Detectable strains

Bacterial strains that have been detected with this kit are listed in Table 3. These strains showed a Ct value of 20 or less when real-time PCR was performed using 10 ng of purified genomic DNA as the template [4)].

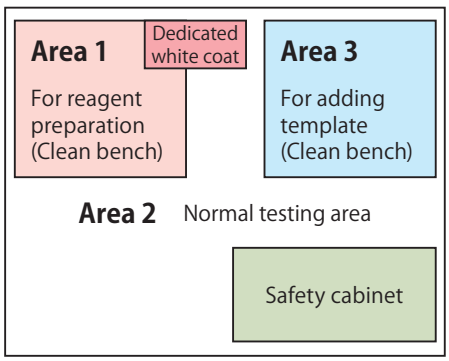
Table 3. Detectable bacterial strains

Bacterial strain[a]	Strain	Source	Accession number	*tuf*-qPCR amplification	*Ct*[b]
Gram negative					
Bacteroidetes					
Chryseobacterium formosense	FI55	Chub mackerel	AB472770	+	16.0
Flavobacterium hercynium	FI48	Spotted mackerel	AB472772	+	17.5
Flavobacterium johnsoniae	JCM 8514		AB472806	+	18.0
Sejongia antarctica	FI18	Spotted mackerel	AB472786	+	17.3
Sphingobacterium kitahiroshimence	FI23	Stone flounder	AB472789	+	16.9
γ -Proteobacteria					
Acinetobacter baumannii	JCM 6841		AB472793	+	15.8
Acinetobacter baumannii	FI63	Flatfish (meita)	AB472767	+	14.3
Aeromonas hydrophila	JCM 1027		AB472795	+	16.5
Aeromonas molluscorum	FI56	Horse mackerel	AB472768	+	15.0
Alteromonas macleodii	JCM 20772		AB472797	+	16.7
Citrobacter freundii	IAM 12471		AB472801	+	15.4
Colwellia aestuarii	FI04	Chub mackerel	AB472771	+	14.1
Enterobacter aerogenes	IAM 1183		AB472802	+	15.8
Erwinia carotovora	IAM 12633		AB472804	+	16.1
Escherichia coli	IAM 1137		AB472805	+	14.2
Klebsiella pneumoniae	IAM 1063		AB472808	+	15.3
Morganella morganii	ATCC 35200		AB472816	+	15.9
Photobacterium phosphoreum	FI59	Horse mackerel	AB472777	+	14.7
Proteus mirabilis	JCM 1669		AB472817	+	17.5
Pseudoalteromonas haloplanktis	FI01	Spotted mackerel	AB472780	+	14.9
Pseudomonas fluorescens	FI28	Stone flounder	AB472781	+	15.8
Psychrobacter immobilis	ATCC 43116		AB472818	+	16.8
Psychromonas arctica	FI26	Chub mackerel	AB472783	+	16.0
Rahnella aquatilis	JCM 1683		AB472819	+	15.9
Raoultella planticola	JCM 7251		AB472820	+	17.5
Salmonella serover Typhimurium	ATCC 13311		AB472822	+	15.1
Schineria larvae	FI13	Spotted mackerel	AB472788	+	15.0
Serratia marcescens	IAM 1104		AB472823	+	16.2
Shewanella frigidimarina	FI20	Spotted mackerel	AB472787	+	15.5
Shewanella japonica	JCM 21433		AB472824	+	15.8
Shewanella putrefaciens	NBRC 3908		AB492873	+	14.4
Vibrio diazotrophicus	FI52	Horse mackerel	AB472791	+	16.2
Vibrio parahaemolyticus	ATCC 17802		AB472827	+	16.3
Xanthomonas euvesicatoria	FI22	Chub mackerel	AB472792	+	15.5

Xanthomonas oryzae	JCM 20241		AB472828	+	19.6
Yersinia enterocolitica	ATCC 9610		AB472829	+	15.9
***α*-Proteobacteria**					
Paracoccus denitrificans	FI34	Spotted mackerel	AB472779	+	17.0
***β*-Proteobacteria**					
Alcaligenes faecalis	JCM 20522		AB472796	+	17.9
Burkholderia caledonica	JCM 21561		AB472799	+	16.3
Gram positives					
Bacilli					
Aerococcus sanguinicola	JCM 11549		AB472794	+	18.4
Bacillus subtilis	IAM 1076		AB472798	+	16.8
Brochothrix thermosphacta	NBRC 12167		AB492875	+	15.3
Carnobacterium divergens	JCM 5816		AB472800	+	15.7
Carnobacterium maltaromaticum	NBRC 15684		AB492874	+	16.2
Enterococcus faecalis	JCM 5803		AB472803	+	14.8
Lactococcus lactis	NRIC 1174		AB472811	+	18.1
Leuconostoc carnosum	JCM 9695		AB472812	+	17.9
Listeria monocytogenes	ATCC 15113		AB472813	+	18.4
Planomicrobium chinense	FI41	Chub mackerel	AB472778	+	16.0
Staphylococcus aureus	ATCC 12600		AB472826	+	16.1
Staphylococcus pasteuri	FI64	Flatfish (meita)	AB472790	+	16.0
Actinobacteria					
Rothia dentocariosa	JCM 3067		AB472821	+	16.1
Rothia nasimurium	FI43	Horse mackerel	AB472784	+	18.2
Salinibacterium amurskyense	FI36	Flatfish (meita)	AB472785	+	17.2

a : Classification was based on the National Center for Biotechnology Information taxonomy.
b : The cycle threshold value (Ct) for the reaction containing 10 ng of genomic DNA per reaction.

X. Appendix 2: Requirements for Preventing Contamination



- Area 1: Area for handling reaction reagents only
  Prepare and dispense real-time PCR reaction solution. (No template DNA is introduced)
- Area 2: Area for normal experimental
  Samples are handled and DNA is prepared.
  Install a safety cabinet as necessary.
- Area 3: Area for handling high concentration DNA
  Add template DNA to the reaction mixture that has already been dispensed.
  Samples to be measured and standard samples are also diluted here.

XI. References

1) Takeo Fujii. Viable Cell Measurement Methods for Aquatic Foods I: Culture Medium Components, Culture Temperature and Plating Methods. *Tokai Suiken Jo*. (1989) **118**: 71-79. (Japanese)

2) Tsubasa Fukuda, Manabu Furushita, and Tusneo Shiba. A Comparison of Viable Cell Counts in Fresh Fish with the Official 35℃ Culture Method and the 20℃ Cell Culture Method. *Journal of National Fisheries University*. (2012) **60**:183-188. (Japanese)

3) Masataka Satomi, Kan Oikawa, and Yutaka Yano. Fisheries Science Series 141: The Quality and Freshness of Seafood and Advanced Preservation Techniques (Edited by Jun'ichi Nakazoe and Hideaki Yamanaka) 6. Microbiological Quality Evaluation. (Japanese)

4) Tanaka Y, Takahashi H, Simidu U, and Kimura B. Design of a new universal real-time PCR system targeting the tuf gene for the enumeration of bacterial counts in food. *J Food Prot*. (2010) **73**: 670-679.

XII. Related Products

TB Green® *Premix Ex Taq*™ GC (Perfect Real Time) (Cat. #RR071A/B)*
NucleoSpin Tissue (Cat. #740952.10/.50/.250)*
Enterobacteriaceae (*rplP* gene) Quantitative PCR Kit (Cat. #RR241A)*
Thermal Cycler Dice™ Real Time System IV with PC (Cat. #TP1010)*

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