iDetect™ Photobacterium damselae Detection kit

Research Use Only
Cat. No. : CG0309

1. Product Indication

iDetect™ Photobacterium damselae Detection kit

2. Intended Use

iDetect™ Photobacterium damselae (P. damselae) Detection kit is a research reagent that qualitatively detects the presence of infection by amplifying the target gene of P. damselae by Real-time LAMP using DNA extracted from samples collected from seawater and marine aquatic products

3. Kit Contents [Packing Unit: 80 tests / kit]

4. Components

Reagents	Descriptions	Volume/ Quantity
2X Rxn buffer	Reaction buffer	800 ㎕/ 1 tube
Primer mix	P. damselae Amplification primer	400 ㎕ / 1 tube
Enzyme mix	Reaction enzyme	80 ㎕ / 1 tube
Positive control	Positive control	60 ㎕ / 1 tube
Negative control	Negative control	60 ㎕ / 1 tube
Cover mix	Mineral oil	800 ㎕ / 1 tube

5. Test Procedure

1) Preparation and Storage

① It is recommended to immediately extract DNA form the collected specimens.

② Specimens stored in containers should be processed within 24 hours during refrigerated storage(2-8°C). For long period of storage, specimens should be stored at -70°C. Specimens stored in shipping containers should be stored at refrigerated storage(2-8°C) until processing can proceed. If a delay in testing or shipping is expected to exceed 2~3 days, specimens can be stored at-70°C.

③ Avoid repeated freeze/thaw samples as it degrades nucleic acids and can reduce sensitivity.

2) Preparation Procedure

① Completely thaw all regents and samples on ice before use.

② Prepare a commercial bacterial DNA extraction kit for extracting DNA

③ Instruments can be used with test
Equipment) AnyDetect (ConnectaGen Inc., Korea)

3) DNA extraction

- Extraction of DNA using the DNA kit should be performed following the manufacturer's instructions.

4) RT-LAMP Reaction Setup

① Prepare the Mastermix according to the following table.

IMPORTANT) This product does not include 1.5ml tube, so it must be prepared separately.

Master mix components	An amount of reaction (㎕)	8 reaction preparation capacity (㎕, 8.5 times)
2X Rxn buffer	10	85.0
Primer mix	4	34.0
Enzyme mix	1	8.5

② Vortex and spin down briefly.

③ Pipet 15㎕ of Mastermix into PCR tube.

④ Pipet 5㎕ of the DNA sample, positive control and negative control to the tube, respectively.

⑤ Add 10㎕ of cover mix to each tube and close the lid.

⑥ Spin-down the tubes

⑦ Install the tubes on AnyDetect and react under the following conditions.

[Ex] Dispensing of 8-strips to amplify target gene in 8 tube reactions

Component	PCR tube No. & amount (㎕)							
	1	2	3	4	5	6	7	8
M.M.	15	15	15	15	15	15	15	15
NC	5	-	-	-	-	-	-	-
PC	-	5	-	-	-	-	-	-
Sample	-	-	5	5	5	5	5	5

M.M. : Amplification master mix
NC : Negative control
PC : Positive control
Sample : Sample DNA

[Reaction condition]

Step	Temperature	Time
1	60℃	3 min
2**	60℃	40 min

** : Turbidity detection step (Scan step)

6. Result Interpretation

1) Set Threshold values, baseline start and end values for each target according to the following table.

Instrument	Target	Threshold	Cut-off
AnyDetect	P. damselae	0.02	40:00

2) Interpretation criteria of the results is in table below.

Results	Tt Values
Positive (+)	T_T *≤ 40:00
Negative (-)	N/D**

*T_T : Threshold time
**N/D : Not detected

3) Result interpretation

Case	PC†	NC†	Target	Results
			P. damselae	
1	+	-	-	P. damselae not detected
2	+	-	+	P. damselae detected
3	-	-	+/-	Invalid / retest
4	+	+	+/-	Invalid / retest
5	-	+	+/-	Invalid / retest

†PC : positive control, NC : negative control

7. Quality Control

1) Confirm the results of positive control and negative control as described below.

2) Retest with the product of the same lot if the controls are not valid. If the repeat result remains invalid, contact the supplier.

Interpretation	Control Type	Results
Valid	Positive control	T_T *≤ 40:00
	Negative control	N/D**
Invalid	Positive control	N/D
	Negative control	T_T ≤ 40:00

*T_T : Threshold time
**N/D : Not detected

8. Storage and Shelf Life

1) All components of the kit are valid for 12 months if reagents are un-opened and stored below -20℃.

2) All components of the kit are valid for 12 days at 4℃ once opened.

9. Warnings and Precautions

1) Repeated testing with the same sample may not be guaranteed statistical significance due to that this kit was designed for a qualitative test.

2) For in vitro diagnostic use only.

3) Please read this user guide carefully before use.

4) Wear disposable gloves, protective clothing, eye protection and masks while handling samples and reagents to avoid contact with the skin and

eyes. If skin and eyes contact occurs, immediately flush with water and seek medical attention.

5) Care should be taken to avoid cross-contamination with amplified products.
6) Clean and disinfect laboratory equipment and space using a disinfectant such as 0.5% sodium hypochlorite, or other suitable disinfectants.
7) Do not reuse of the amplified product after the reaction, and dispose of waste in a designated place.
8) All test samples shall be regarded as infectious substances. The operation of sample and waste for each laboratory shall meet the requirements of relevant laws and regulations.
9) Improper storage may hinder the ability of assay to detect.
10) Do not use a kit after its expiration date, and do not mix reagents from different lots or the same lot with other packaging.
11) The results of this kit should not be used as the sole basis for treatment or other management decisions. Results must be combined with clinical observations based upon other testing methods or expert judgements.

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