

foodproof[®] SL Shrimp and Crab Species Detection Kit

Revision A, March 2024

PCR kit for the qualitative detection of Shrimp and Crab species DNA using real-time PCR instruments.

Product No. KIT230229

Kit for 50 reactions for a maximum of 48 samples

Store at -15 to -25 °C

For food testing purposes.

FOR *IN VITRO* USE ONLY



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1. Introduction

To assure a high level of food and feed safety, accurate animal species identification and the detection of adulterants are two of the greatest challenges facing food and feed products companies today. Therefore, the need for scientifically valid species identity methods is increasingly important. Although a number of traditional morphological, microscopic and chemical methods have commonly been used for species identity testing, technologies using DNA offer reliable alternative methods that can provide increased precision in differentiating closely related species, as well as identifying intentional and accidental adulterants and contaminants.

The Animal Species detection kits are designed for use by food and feed producers, dairies, marketers of these products, as well as regulators and auditors of final food and feed quality and safety. It is also intended to be used to verify that ruminant feed and feed supplements are properly labeled and do not contain ruminant materials.

2. Intended Use

The foodproof® SL Shrimp and Crab Species Detection Kit is designed to detect the specific gene for shrimp and crab species in feed, food, and raw material. This kit provides a real-time PCR Master Mix with enzyme components and the specific primer/probe set for rapid testing by real-time PCR assay, as well as the Internal Control (IC) system for reliable results.

3. Principle of PCR detection

The foodproof SL Shrimp and Crab Species Detection Kit is a qualitative, duplex real-time PCR test, for the detection of a shrimp and crab specific gene and the Internal Control (IC) using specific primers and probes labeled with the fluorescent dyes. The target sequences are detected through the FAM and HEX (VIC) channels, respectively.

The primer and probe mixture provided exploits the so-called TaqMan® principle. During PCR amplification, forward and reverse primers hybridize to the target DNA. A fluorogenic probe is included in the same reaction mixture, which consists of an oligonucleotide labeled with a 5'-reporter dye and a downstream 3'-quencher. During PCR amplification, the probe is cleaved and the reporter dye and quencher are separated. The resulting increase in fluorescence can be detected on a range of real-time PCR platforms. The monitoring of the fluorescence intensities during the real-time PCR allows the detection of accumulating product without re-opening the reaction tubes after the PCR run, such as electrophoresis.

The kit minimizes contamination risk and contains all reagents needed for detection (except for PCR-grade H₂O).

3.1 Internal Amplification Control

This kit contains the Internal Control (IC) as PCR inhibition Control. The IC allows the user to determine and control possible PCR inhibition. The IC reagents are included in the primer/probe Mixture and the IC is co-amplified with target DNA from the sample. The results can be visualized in the HEX (VIC) channel.



4. Contents

This kit is intended for 50 reactions, including controls.

Table 1: Kit Contents

| Reagent | Cap Label | Volume | Description |
|-----------------------------|-----------|--------|--|
| 2x real-time PCR Master Mix | 2xM | 500 µL | Buffer containing dNTPs, MgCl ₂ and Taq DNA polymerase |
| Primer / Probe Mix | P | 200 µL | Primer/ probe mixture: <ul style="list-style-type: none"> • Shrimp and Crab specific primer and probe • IC-specific primer and probe • IC DNA |
| Control DNA | C | 50 µL | Positive control DNA |

5. Additional Materials, Reagents and Devices Required

- Disposable powder-free gloves and laboratory coat
- Pipettors (0.5 to 10 µL, 2 to 20 µL, 20 to 200 µL, 200 to 1,000 µL)
- Sterile aerosol-barrier pipette tips
- Ice or benchtop cooler
- Vortex mixer
- Clean bench area or PCR box
- Tabletop centrifuge with rotor for 2 mL reaction tubes
- Real-time thermal cycler with FAM and HEX (VIC) detection channels
- Disposable polypropylene microtubes for PCR
- PCR-grade H₂O
- For DNA Extraction: foodproof Sample Preparation Kit

6. General precautions

- Store extracted positive material (samples, controls and other amplicons) away from all other reagents and add to the reaction mix in a separate area.
- Thaw all components thoroughly on ice before starting the experiment.
- When thawed, mix the components and centrifuge briefly.
- Do not pipette by mouth.
- Do not eat, drink, smoke, apply cosmetics or handle contact lenses in laboratory work areas.
- Do not use a kit beyond its expiration date.
- Safety Data Sheets (SDS) can be found at www.hygiena.com/documents.
- Use disposable gloves, laboratory coats and eye protection while handling samples and reagents. Thoroughly wash hands afterward.
- Dispose of all samples and unused reagents in compliance with local regulations.
- Specimens should be considered potentially infectious and handled in a biological cabinet in accordance with Biosafety Level 2 or other appropriate biosafety practices.



- Clean and disinfect all sample or reagent spills using a disinfectant such as 0.5% sodium hypochlorite or other suitable disinfectant.
- Avoid contact of specimens and reagents with the skin, eyes and mucosa. If contact occurs with skin, eyes or mucosa, immediately flush with water and seek medical attention.
- Use of this product should be limited to personnel trained in laboratory DNA amplification techniques.
- To avoid carry-over contamination with PCR product or control DNA, please note the following points:
 1. Be careful not to contaminate the Primer/Probe Mixture and 2x real-time PCR Master Mix with other PCR products or Control DNA through pipetting. To prevent contamination, the use of aerosol-barrier tips is recommended.
 2. Open and close all sample tubes carefully. Avoid splashing or spraying PCR samples.
 3. It is important to have designated lab areas where PCR reactions are set up, preferentially separated in space from the areas where PCR reactions are analyzed.
 4. The laboratory process must be one-directional; it should begin in the Extraction Area and move to the Amplification and Detection Area. Do not transport samples, equipment and reagents to the areas where you performed previous steps.

7. Sampling and handling

7.1 Sample Collection

Various processed food, raw material, feed and seed samples are routinely examined.

7.2 Sample Storage

The assay sensitivity can be reduced if you routinely freeze the samples before testing or store them for an extended period of time. Avoid repeated freezing and thawing of samples, which may lead to DNA degradation and decreased sensitivity.

7.3 Nucleic Acid Extraction

Carry out DNA isolation according to the extraction kit's product instructions. For more information, please see www.hygienea.com.

8. Protocol

8.1 DNA Isolation

Hygienea® Diagnostics provides sample preparation kits suitable for all kinds of foods and raw materials (See 5. "Additional Required Materials, Reagents and Devices").

8.2 Preparing the PCR

To prevent the risk of contamination with foreign DNA, we recommend that all experiment steps be performed in a PCR cleanroom or separated environment area. Aerosol-barrier pipette tips are recommended for each step.

8.2.1 Thawing the Kit Components

The use of ice or a benchtop cooler is recommended during experiments to maintain enzyme activity.



8.2.2 Prepare Reaction Master Mix

Each reaction has a total volume of 20 µL; the volume of the DNA sample is 6 µL.

1. Prepare the reaction mixture according to Table 2 below.

Table 2: PCR reaction mixture

| Composition | Volume |
|-----------------------------|--------|
| Primer / Probe Mixture | 4 µL |
| 2x real-time PCR Master Mix | 10 µL |
| Total | 14 µL |

2. Add 6 µL of extracted DNA sample into the tube.

8.2.3 Prepare Control Amplification Reactions

CONTROL +

- Positive control amplification: Add 6 µL of Control DNA instead of sample DNA.

CONTROL -

- Negative control amplification: Add 6 µL of PCR-grade H₂O instead of sample DNA

8.2.4 Mixing

Mix the reagents in the PCR reaction tubes by tapping a minimum of 5 times. Briefly centrifuge the tubes to remove any air bubbles or drops inside the cap.

8.3 Amplification

- Program your real-time PCR instrument according to the manufacturer’s manual.
- Create a temperature-time profile on your instrument as follows in Table 3.

Table 3: Temperature Time Profile

| Temperature | Time | Cycle |
|-------------|------------|-------|
| 95 °C | 10 min | 1 |
| 95 °C | 15 seconds | 35 |
| 61 °C* | 40 seconds | |

*Detect the fluorescence at this step.



9. Data analysis

The fluorescence curves are analyzed in FAM and HEX (VIC) fluorescence detection channels (see Table 4). You can predict the presence or absence of the target gene in your samples by analyzing the real-time PCR result.

Table 4: Specific Detection on Fluorescence Channel

| Target Gene | Fluorophore |
|-------------------------------|-------------|
| Shrimp and Crab specific gene | FAM |
| IC | HEX (VIC) |

9.1 Interpretation of Results

- The signal is considered to be positive if the corresponding fluorescence accumulation curve crosses the threshold line.
- Results are accepted as relevant if both positive and negative amplification controls pass.
- **IC:** When amplifying a target sample with a high copy number, the IC may not produce an amplification plot. This does not invalidate the test and should be interpreted as a positive experimental result.

Table 5: Interpretation of Results

| | Positive Control | Negative Control | FAM | JOE (HEX or VIC) | Interpretation |
|--------|------------------|------------------|-------------------------------|------------------|---|
| | | | Shrimp and Crab specific gene | IC | |
| Case 1 | + | - | + | + | The shrimp- and crab-specific gene is detected in a sample. |
| Case 2 | + | - | + | _* | The shrimp- and crab-specific gene is detected in a sample. |
| Case 3 | + | - | - | + | The shrimp- and crab-specific gene is not detected in a sample. |
| Case 4 | + | - | - | - | Invalid result/retest. |
| Case 5 | + | + | +/- | +/- | |
| Case 6 | - | + | +/- | +/- | |
| Case 7 | - | - | +/- | +/- | |

*Detection of the Internal Amplification Control in the respective channel is not required for a positive result. A high copy number of the target gene can lead to reduced or absent Internal Amplification Control signal.



10. Troubleshooting

| Situation | Possible cause | Recommendation |
|--|--|---|
| Negative control samples are positive. | Carry-over contamination | <ul style="list-style-type: none"> • Exchange all critical solutions. • Repeat the analysis of all tests with fresh aliquots of all reagents. • Take measures to detect and eliminate the source of contamination. |
| No signal is detected for amplification positive controls. | Incorrect programming of the real-time PCR instrument. | <ul style="list-style-type: none"> • The PCR should be repeated after checking the programming of instruments, storage conditions and the expiration date. |
| | The kit reagents have expired. | |
| | Kit components have not been stored according to the manufacturer's instructions. | |
| No signal is detected for IC on HEX (VIC) channel and shrimp- and crab-specific gene on FAM channel. | <ul style="list-style-type: none"> • Incorrect PCR reaction • Pipetting errors • Omitted reagents | <ul style="list-style-type: none"> • The PCR should be repeated after checking for correct pipetting scheme and reaction setup. |
| | PCR inhibitors are present at a high concentration. | <ul style="list-style-type: none"> • DNA extraction should be repeated. |

11. Stability and Storage

Store the kit at -15 to -25 °C through the expiration date printed on the label.

12. Specifications

- **Sensitivity**
50 GE Limit of detection (LOD)
- **Specificity**
100% exclusivity for about 100 non-specific species DNAs

13. Quality control

In compliance with the Federal State Institution of Science “Central Research Institute of Epidemiology” ISO 13485 – certified Quality Management System, each lot of foodproof® SL Shrimp and Crab Species Detection Kit has been tested against predetermined specifications to ensure consistent product quality.



14. Ordering information

| Product | Order No. | # Tests |
|--|-----------|--------------|
| foodproof SL Shrimp and Crab Species Detection Kit | KIT230229 | 50 reactions |
| foodproof Sample Preparation Kit III | KIT230174 | 50 reactions |

15. Supplementary Information

15.1 Ordering Information

Hygiena Diagnostics offers a broad range of reagents and services. For a complete overview and for more information, please visit our website at www.hygiena.com.

15.3 Trademarks

foodproof®, **microproof®**, **vetproof®**, **ShortPrep®**, **StarPrep®**, **RoboPrep®** and **LyoKit®** are registered trademarks of Hygiena Diagnostics GmbH. **Hygiena®** is a registered trademark of Hygiena. Other brand or product names are trademarks of their respective holders.

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15.4 Contact and Support

If you have questions or experience problems with this or any other product of Hygiena Diagnostics GmbH, please contact our Technical Support staff (www.hygiena.com/support). Our scientists commit themselves to providing rapid and effective help. We also want you to contact us if you have suggestions for enhancing our product performance or using our products in new or specialized ways. Such customer information has repeatedly proven invaluable to us and the worldwide research community.

15.5 Reference Number

The reference number and original Hygiena Diagnostics GmbH article number: Z 730 08

16. Change Index

Version 1, April 2018

First version of the package insert.

Revision A, March 2024

Rebranding and new layout.

Z 730 08 20 -> INS-KIT230229-RevA



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