

HUNTER ALL AS

Cat. No. F02-03-1115R

Real-time PCR test kit for qualitative detection of
Animal Specific genetic sequences in food

Includes main components for 100 PCR amplification reactions for
Roche LightCycler® Real-Time PCR System



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HAI KANG LIFE CORPORATION LIMITED

PLEASE READ THROUGH THE ENTIRE PROTOCOL BEFORE
STARTING.

1. KIT COMPONENTS

- 3 x 400 µl All AS Real-time Mastermix (store at -20°C)
- 1 x 50 µl Positive Control (store at -20°C)

Storage conditions

The mastermixes and positive controls should be stored at -20°C. Real-time mastermixes contain light-sensitive components, it is recommended to wrap the tubes with aluminum foil.

Note:

Please aliquot the mastermix solutions into appropriate volume according to your test frequency, in order to minimize repeated freeze and thaw cycles. Frequent thawing and freezing may inactivate some kit components.

2. PROCEDURE

Note: Thaw kit reagents just before use. Mix thawed reagents thoroughly. Do not vortex the enzyme-containing mastermix. Only thaw as many PCR mastermix tubes as are required.

- Thaw PCR mastermix solutions on ice. Make sure the tubes are protected from light.
- Set up mastermix and PCR components according to the table below:

| Component | Volume per reaction (µl) |
|------------------------|--------------------------|
| Real-time mastermix | 11.2 |
| DNA template | X |
| Double-distilled water | 8.8 - X |
| Total Volume | 20 |

Note: For each real-time PCR assay, positive controls (use 2 µl) and negative control (use 2 µl water) should be included. Test samples should be prepared in duplicate PCR reactions.

- Use appropriate volume of DNA template and double-distilled (dd) water. Use ~100-200 ng DNA template for each PCR reaction. (DNA concentration should have been determined spectrophotometrically after DNA extraction steps.)

Note: Use real time PCR tubes and consumable recommended for your real-time PCR equipment.

- When all PCR reactions are set up, load all PCR tubes into the real-time PCR equipment using cycling conditions shown below:

| | Data Collection Points (FAM Filter / 530nm) | | |
|-----------|--|------------|---|
| 1 Cycle | 50°C | 2 Minutes | - |
| | 95°C | 10 Minutes | - |
| 40 Cycles | 95°C | 15 Seconds | - |
| | 60°C | 60 Seconds | □ |

(Take readings at point □)

3. DATA COLLECTION AND ANALYSIS

Detailed explanations of the basic and advanced operating procedures should be provided with your real-time PCR equipment. This kit is optimised using the Roche LightCycler® 2.0 Real-Time PCR System.

The calculation of the most appropriate cut-off limit to adequately discriminate between genuine positive and negative samples is a relatively straightforward procedure. However, care must be taken to calculate the cut-off limit for each set of experiments that are conducted.

In general, Ct values of 40 in replicate samples are presumed to be negative for the template of interest. If one replicate gives a Ct <40 while another gives a Ct of 40, the result is inconclusive and the analysis should be repeated. If all replicates consistently give Ct <40, the sample is positive for the presence of template.

If you require more detailed analysis information, please contact Hai Kang Life Corporation Limited for technical assistance.

4. TECHNICAL ASSISTANCE

Our technical staff will provide technical assistance you may need in using this kit. Simply call +(852) 2111 2123 during office hours:

Monday – Friday: 9:00am to 5:30pm

Saturday: 9:00am to 1:00pm

A recorded message (in English, Cantonese or Putonghua) may be left outside office hours. Alternatively, you are welcomed to contact our technical staff by fax or email.

Fax: +(852) 2111 9762

Email: technical@haikanglife.com

5. WARRANTIES AND LIABILITIES

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