GMO
Watcher 1.0
Cat. No. F01-01-1111

Gel based PCR test kit for the qualitative detection of
35S, NOS and U-Plant genetic sequences in food

Includes main components for 300 PCR amplification reactions

Rev. 1
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HAI KANG LIFE CORPORATION LIMITED

PLEASE READ THROUGH THE ENTIRE PROTOCOL BEFORE STARTING.

1. KIT COMPONENTS

- 2 x 275 µl 35S Mastermix (store at –20°C)
- 2 x 275 µl NOS Mastermix (store at –20°C)
- 2 x 275 µl U-Plant* Mastermix (store at –20°C)
- 1 x 90 µl Positive Control (store at –20°C)
- 1 x 30 µl Taq Polymerase (store at –20°C)

*For detection of a universal plant gene in order to verify the presence of plant DNA.

Storage conditions
Store Mastermix, Positive Control and Taq polymerase at -20°C.

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.

1. Thaw PCR mastermix solutions on ice.
2. Set up mastermix and PCR components according to the table below:

Component	**35S, NOS or U-Plant**
	Volume Per reaction (µl)
Mastermix	5.1
DNA template	X
Double-distilled water	14.8 – X
Taq Polymerase	0.1
Total Volume	20

3. Use appropriate volume of DNA template and double-distilled (dd) water. For 35S, NOS or U-Plant, use ~200 ng DNA template for each PCR reaction. (DNA concentration should have been determined spectrophotometrically after DNA extraction steps.)

Note:
- For each 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.
- It is advisable to run samples in duplicate to ensure reliability of results.
- Spiking Control: 1 µl Positive Control can be spiked into test sample to check whether the test sample contains PCR inhibitory substances.

4. When all PCR reactions are set up, load all PCR tubes into the PCR thermal cycler and use the cycling conditions shown below:

1 Cycle	94°C	10 Minutes
40 Cycles	94°C	30 Seconds
	57°C	30 Seconds
	72°C	30 Seconds
1 Cycle	72°C	10 Minutes

3. DATA ANALYSIS AND INTERPRETATION

Expected product sizes:

35S	162 bp
NOS	146 bp
U-Plant	584 bp

Spiking control
Negative PCR result may be due to a few scenarios: 1. absence of detected sequence in the sample; 2. presence of detected sequence below limit of detection; 3. presence of PCR inhibitory substances. The purpose of spiking control is to verify whether the test sample contains substances, which may affect PCR reactions. If no band is visible on agarose gel when the test sample is spiked with the positive control, the test sample is highly likely to contain PCR inhibitory substances, and the result should NOT be taken as negative. Repeated extraction and PCR of the sample will be required.

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** for assistance during our office hours:

Monday – Friday: 9:00 a.m. to 5:30 p.m.
Saturday: 9:00 a.m. to 1:00 p.m.

A recorded message (in English, Cantonese or Putonghua) may be left at other times. Alternatively, you are welcome to contact our technical staff by fax or email.

Fax: +(852) 2111 9762
Email: technical@haikanglife.com

5. WARRANTIES AND LIABILITIES