

SN

**INDUSTRY STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA**

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**PCR protocol for detection of genetically modified plant
ingredients in feed**

饲料中转基因植物成份PCR检测方法

(English Translation)

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1 Scope

This standard specifies the qualitative real-time fluorescent PCR detection method, for transgenic plant components in feed.

This standard is applicable to the qualitative detection of genetically modified plant components, such as soybean, corn, rice, rape, cotton, lucerne, wheat in feed, as well as the identification of related lines.

This standard is also applicable to the qualitative detection of the genetically modified components of distiller dried grains with soluble, as well as the identification of related lines.

2 Normative references

The following documents are essential to the application of this document. For the dated documents, only the versions with the dates indicated are applicable to this document;

for the undated documents, only the latest version (including all the amendments) is applicable to this standard.

GB/T 6682 Water for analytical laboratory use - Specification and test methods

GB/T 19495.2 Detection of genetically modified organism and derived products -
General requirements for laboratories

GB/T 19495.7 Detection of genetically modified organisms and derived products -

Methods for sampling and sample preparation

3.1 Terms and definitions

The following terms and definitions apply to this document.

3.1.1

Feed

The general term for the food of animals, which are raised by all people. The general feed, in the narrower sense, mainly refers to the food of animals, which are raised in agriculture or animal husbandry.

3.1.2

Real-time fluorescent polymerase chain reaction

Fluorescent groups are added to the polymerase chain reaction system, the entire PCR process is monitored in real time by the accumulation of fluorescent signals, the intensity of fluorescent signals directly reflects the number of templates.

3.2 Abbreviations

The following abbreviations apply to this document.

Acp1: acid phosphatase 1

BAR: phosphinothricin acetyltransferase gene

BnACCG8: acetyl-CoA carboxylase gene bp: base pair

Btc: the construct specific DNA sequence of transgenic rice TT51-1 and Bt shanyou

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CaMV35S: 35S promoter from cauliflower mosaic virus

CP4-EPSPS: 5-enolpyruvylshikimate-3-phosphate synthase gene

CryIA(b): The cryIA (b) gene of *Bacillus thuringiensis* subsp [a synthetic gene encoded the first 648 amino acids, insecticidal-active truncated product identical to that of cryIA (b) gene of *Bacillus thuringiensis* subsp, Kurstaki strain HD-1]

CryIA(c): The cryIA (c) gene of *Bacillus thuringiensis* [a synthetic gene encoded the 29 to 613 amino acids, insecticidal-active truncated product identical to that of cryIA (c) gene of *Bacillus thuringiensis*]

CryIA (b) /CryIA(c): Fused gene of *Bacillus thuringiensis* insecticidal protein cryIA (b) and cryIA (c)

Ct value: The number of cycle, that the fluorescent signal in each reaction tube will go through, when it reaches the set threshold (cycle threshold)

CTAB: cetyltrimethylammonium bromide dATP: deoxyadenosine triphosphate

4 Principles

Real-time fluorescence quantitative PCR technology refers to the method of adding fluorescent groups to the PCR reaction system, using the accumulation of fluorescent signals to monitor the entire PCR process in real time, finally quantitatively analyzing the unknown template through the standard curve.

During PCR amplification, a specific fluorescent probe is added at the same time as a pair of primers. The probe is an oligonucleotide; the two ends are respectively labeled with a reporter fluorescent group and a quenching fluorescent group. When the probe is intact, the fluorescent signal emitted by the reporter gene is absorbed by the quenching fluorescent group. During PCR amplification, the 5'-3' exonuclease activity of Taq enzyme cleaves and degrades the probe, making the reporter fluorescent group be separated from the quenching fluorescent group, so that the fluorescence monitoring system can receive the fluorescent signal, that is, a fluorescent molecule is formed every time a DNA chain is amplified; thereby, it achieves the complete synchronization between the accumulation of the fluorescent signal and the formation of the PCR product.

5.1 Main equipment

Real-time fluorescent PCR instrument, ice maker, nucleic acid protein analyzer or UV spectrophotometer, constant temperature water bath, centrifuge, mortar and crushing device, vortex shaker, micropipette (2.5 μ L, 20 μ L, 200 μ L, 1000 μ L).

5.2 Main reagents

Unless otherwise specified, the grades of reagents used in all tests shall be of analytical pure or biochemical reagents, which do not contain DNA and DNase. The water used in the test shall meet the specifications of grade 1 water in GB/T 6682; all reagents shall be packaged in containers, which are contaminated by DNase.

5.2.1

CTAB extract

20 g/L CTAB, 1.4 mol/L NaCl, 0.1 mol/L Tris-HCL, 0.02 mol/L

Na₂-EDTA, pH 8.0.

5.2.2

CTAB precipitation solution

5 g/L CTAB, 40 mmol/L NaCl.

5.2.3

Proteinase K solution

20 mg/mL.

5.2.4

RNase A

100 mg/mL.

5.2.5

NaCl solution

1.2 mol/L.

There are many kinds of feeds; the ingredients are extremely complex. In addition to various plant ingredients, it may also contain a variety of premixes, such as protein meal, bone meal, amino acids, vitamins, antibiotics (or veterinary drugs), minerals, antifungal agents, insect repellents, colorants, seasonings, binders, fermentation products. These salts, sugars, pigments and other chemicals, in the feed sample, will affect the subsequent extraction of genomic DNA; it may even inhibit subsequent PCR reactions, etc. They shall be removed, by washing and other methods. Take 200 mg of crushed feed sample; put it into a 2 mL centrifuge tube; add 1.5 mL of double-distilled water;

invert upside down; vortex to mix it; centrifuge it at 12000 g for 5 min; discard the supernatant; add 1.5 mL of double-distilled water to the precipitate; repeat the above washing process 3 ~ 5 times. The final sediment is used for the extraction of genomic DNA.

8.2.1 CTAB method

The CTAB extraction method is as follows:

a) Add 1000 μ L of CTAB extraction buffer and 2 μ L of proteinase K solution, into the above 200 mg pretreated sample. Incubate it at 65 °C, for 60 min. Invert and mix 3 ~ 5 times during this period. OR incubate it at 65 °C overnight. Centrifuge it at 12000 g for 10 min. Transfer the supernatant to another clean 2 mL centrifuge tube.

b) Add the same volume of chloroform as the supernatant. Invert and mix it.

Centrifuge it at 12000 g for 10 min. Transfer the supernatant to another 2 mL centrifuge tube.

c) Add 2 times the volume of CTAB precipitation solution. Invert and mix it. Let it stand at room temperature for 30 minutes. Centrifuge it at 12000 g for 10 minutes.

Discard the supernatant.

d) Add 500 μ L of sodium chloride solution to the sediment, to dissolve the sediment.