Translated English of Chinese Standard: SN/T1196-2012
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SN

Industry Standard
of the People's Republic of China

SN/T 1196-2012

Replacing SN/T 1196-2003

Detection of genetically modified components – Maize test methods

转基因成分检测 玉米检测方法

Issue on: December 12, 2012 **Implemented on: July 1, 2013**

Issued by: State Administration of Quality Supervision and Inspection and Quarantine of the People's Republic of China

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Foreword

This Standard was drafted in accordance with rules given in GB/T 1.1-2009.

This Standard replaces SN/T 1196-2003 "GMO maize Qualitative PCR detection methods". Compared with SN/T 1196-2003, the main technical changes of this Standard are as follows:

- MIDOFY the Chinese and English name of the standard;
- ADD the scope of identifying maize lines, AMEND qualitative detection of genetically modified lines of MON863, NK603, TC1507, MON88017, 59122, MIR604, MTR162, DBT418, MON89034, LY038, ES3272, Bt10, and DP98140.
- ADD 2nd method - real-time PCR method.

This Standard references PCR detection method of genetically modified maize in ISO standards and the EU Joint Research Centre - Community reference laboratory for GM food and feed Biotechnology & GMOs Unit. In the 1st method – common PCR method, ADD qualitative detection of genetically modified lines of MON863 NK603, TC1507, and Bt10. ADD 2nd method - real-time PCR method.

This Standard is proposed and managed by the National Certification and Accreditation Administration Committee.

Drafting organizations of this Standard: Liaoning Entry-Exit Inspection and Quarantine Bureau, Chinese Academy of Inspection and Quarantine, Shandong Entry-Exit Inspection and Quarantine Bureau, Fujian Entry-Exit Inspection and Quarantine Bureau, and Shenzhen Entry-Exit Inspection and Quarantine Bureau.

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The previous standard replaced by this Standard is:

- SN/T 1196-2003.

Detection of genetically modified components – Maize test methods

1 Scope

This Standard specifies the PCR method and real-time fluorescence PCR method of detecting genetically modified components in maize and maize-processed products.

The screening detection of this Standard applies to qualitative detection of genetically modified components in maize and maize-processed products.

Identification detection of this Standard applies to qualitative detection of genetically modified components of maize lines MON810, Bt11, Bt176, T14/T25, CBH351, GA21, MON863, NK603, TC1507, MON88017, 59122, MIR604, MIR162, DBT418, MON89034, LY038, ES3272, Bt10, and DP98140.

2 Normative references

The following documents for the application of this document are essential. Only those dated documents are applicable to this document. For undated references, the latest edition (including any amendments) applies to this document.

GB/T 6682	Analytical laboratory use - Specification and test methods
GB/T 19495.1	Detection of genetically modified organisms and derived products. General requirements and definitions
GB/T 19495.2	Detection of genetically modified organism and derived products. General requirements for laboratories
GB/T 19495.3	Detection of genetically modified organisms and derived products. Nucleic acid extraction
GB/T 19495.7	Detection of genetically modified organisms and derived products. Gene-chip detection

6.2.15 10 X loading buffer: 0.25% bromophenol blue, 40% sucrose.

6.2.16 RNA enzyme (10μg/mL).

6.2.17 UNG enzyme (Uracil N-glycosylase).

6.3 Instruments

Solid grinder and mortar; high-speed refrigerated centrifuge; desktop mini centrifuge, mini individual centrifuge; bath incubator, incubator, constant temperature incubator; balance: sensitivity of 0.001 g; autoclave; temperature oven; distilled water maker or double water heater; refrigerator, freezers; ice maker; vortex oscillator; microwave oven; Cycler; electrophoresis; PCR clean bench; nucleic acid protein analyzer; micropipette (0.1μL - 2μL, 0.5μL - 10μL, 2μL - 20μL, 10μL - 100μL, 20μL - 200μL, 200μL - 1000μL); gel imaging system; centrifuge tube: 1.5 mL - 5 mL; PCR reaction tube: two specifications of 200μL, 500μL.

6.4 Detection steps

6.4.1 Settings of contrast

Contrast shall be set in accordance with provisions in GB/T 19495.2 during the detection procedure.

DNA contrast of negative target: It shall not contain DNA fragment of exogenous target nucleic acid sequence.

DNA contrast of positive target: It can be the reference DNA, or the DNA extracted from traceable reference material, or the DNA extracted from positive sample (or organism) which contains known sequences.

Contrast of amplification reagent: All reaction reagents except the DNA template of test samples. USE the same volume of water (excluding the nucleic acid) to substitute template DNA in the PCR reaction system.

6.4.2 Extraction of template DNA

WEIGH 1 g of powder sample into 10 mL centrifuge tube; ADD 5 mL of CTAB lysis buffer (including appropriate amount of RNA enzymes); MIX well; at 60ºC water bath SHAKE for 1

7.4.7.2 Relation between Ct value and DNA concentration

When Ct value is greater than or equal to 40, there is no amplification of the target DNA in PCR procedure; if Ct value is between 36 - 40, and difference between each value of parallel samples is very large, it indicates that there is little amount of target DNA in the PCR reaction system; it shall appropriately increase the template amount.

7.5 Quality Control of real-time fluorescence PCR detection

Amplification Reagent Contrast: Detected Ct value of exogenous gene is greater than or equal to 40; detected Ct value of endogenous gene is greater than or equal to 40.

Negative target DNA contrast: Detected Ct value of exogenous gene is greater than or equal to 40.

Positive target DNA contrast: Detected Ct value of exogenous gene is smaller than or equal to 34.

If there is one item of above indicators not compliant, it shall repeat the real-time fluorescence PCR amplification.

7.6 Selection of screening test and identification test

For detection of genetically modified component in maize sample, it may be performed according to the contents of Appendix B. Firstly SCREEN and TEST CaMV 35S, NOS, NPTII, PAT, BAR and CryIA(b) genes, if the screening test result is negative, then directly REPORT the test result.

If the screening test result is positive, then it needs further identification detection of line specificity gene of MON810, Bt11, Bt176, T14/T25, CBH351, GA21, MON863, NK603, TC1507, MON88017, 59122, MIR604, MIR162, DBT418, MON89034, LY038, ES3272, Bt10 and DP98140, so as to determine the line of genetically modified maize.

7.7 Judgment of results

If the detection value Ct of exogenous gene of test sample is greater than or equal to 40, the detection value Ct of endogenous gene of test sample is smaller than or equal to 24, and the setting contrast result is normal, then it can be judged that XXX gene is not

References

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[4] Event-specific method for the quantitation of maize line MON 863 using real-time PCR, EU-CRL.

[5] Event-specific Method for the Quantification of Maize Line MON 89034 Using real-time PCR, EU-CRL.

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