POROČILO O PREISKUSU
ANALYSIS REPORT

NACIONALNI INŠTITUT ZA BIOLOGIJO
NATIONAL INSTITUTE OF BIOLOGY
ODDELEK ZA BIOTEHNOLOGIJO IN SISTEMSKO BIOLOGIJO
DEPARTMENT OF BIOTECHNOLOGY AND SYSTEMS BIOLOGY
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SLOVENSKA AKREDITACIJA LP-028

Report No.: GMO 031/12

DETECTION OF GENETICALLY MODIFIED ORGANISMS

Customer:	Farmland a.d. Nova Topola b.b. 78418 Nova Topola Bosni and Hercegovina
Contact person:	Mr. Nenad Pavić
Sample number:	G031/12
Customer's designation of the sample:	**Milk 23.05.2012**
Date of sample receival:	23.5.2012
Sample specifications:	Milk
Sample quantity:	1L
Analysis order:	5-plex screening analysis
Date of analysis:	23.5.2012 - 24.5.2012

DESCRIPTION OF PERFORMED ANALYSIS:

Presence of plant specific DNA in the sample is confirmed by amplification of plant specific gene by Real-time PCR or by PCR.

According to the order, analysis for the presence of screening elements, specific genetic sequences of each genetically modified organism and for amount of genetically modified organisms in the sample is performed.

For the presence of screening elements which indicates the presence of genetically modified organisms in the sample, section of 35S promoter from cauliflower mosaic virus, section of NOS terminator from bacteria *Agrobacterium tumefaciens*, pat and bar genes from bacterial genus *Streptomyces* and CTP2-CP4-EPSPS gene construct are amplified. Presence of the 35S promoter and/or NOS terminator and/or bar gene and/or pat gene, which have been introduced in different genetically modified plants, are strong indicators of genetic changes, but are not an absolute proof, because these sequences can be present naturally. Result could be positive due to the infection of the sample with cauliflower mosaic virus or infection with bacteria *Agrobacterium tumefaciens* or infection with bacteria *Streptomyces hygroscopicus* or infection with bacteria *Streptomyces viridochromogenes*. Further analysis of specific genetic sequences of each genetically modified organism should be performed at positive result with screening elements to identify source of 35S promoter, NOS terminator, pat and bar gene and CTP2-CP4-EPSPS gene construct. False positive result of the presence of 35S promoter can be checked by the amplification of cauliflower mosaic virus (CaMV) specific sequences.

For determination of each genetically modified organism, genetic sequence, specific for each genetically modified organism, is amplified. Those sequences do not occur naturally, therefore the positive result signifies the presence of certain genetically modified organism in the sample. The amount of each genetically modified organism in the sample is established by comparison with standard reference material with known concentration of genetically modified organism.

Practical limit of detection/quantification in the sample means amount of genetically modified organism which could be detected/quantified in the tested sample. Practical limit of detection/quantification is determined on the basis of amount of plant specific DNA in the sample and detection/quantification limit of the tested method.

In the sample listed test methods were performed in accordance with standard operating procedures.

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TEST METHODS PERFORMED IN ACCORDANCE WITH STANDARD OPERATION PROCEDURES:

02G-Pos21	Isolation and purification of DNA with GeneSpin or NucleoSpin Food kit
02G-Pos53	Quantitative determination of maize reference gene (High Mobility Group protein a, HMGa) by Real-time PCR
02G-Pos65	Qualitative determination of p35S, tNOS, bar, pat and CTP2-CP4-EPSPS by Real-time PCR

RESULTS:

5-plex screening analysis

Amplicon	*Result*	*Limit of detection (LOD) of the method*
HMGa(maize)	DNA not detected	2 copies of target DNA
CTP2-CP4-EPSPS	not detected	15 copies of target DNA
pat	not detected	15 copies of target DNA
bar	not detected	15 copies of target DNA
tNOS	not detected	15 copies of target DNA
p35S	not detected	15 copies of target DNA

Limit of detection was determined on certified reference material.

Practical limit of detection /quantification:

Transgenic maize lines: the amount of extracted target DNA was insufficient for determination of practical limit of detection in the sample.

This report is written in two copies from which one is given to the customer. One copy of this report is kept in the archives of the Department of Biotechnology and Systems Biology on National Institute of Biology. Documentation about the sample will be kept in the archives for 5 years. Results of testing are valid only for the material tested in the laboratory. This report can not be reproduced partially without a written consent of our laboratory.

Analyzed by:

Dejan Štebih

Signature:

Head of GMO testing:

Prof. dr. Jana Žel

Signature:

Ljubljana, 24.5.2012

Stamp of NIB