Bundesamt für
Verbraucherschutz und
Lebensmittelsicherheit

# Sampling and analysis guidelines for the detection of genetically modified flax

This document was developed by the § 64 LFGB working group “Development of methods to identifying foodstuffs produced by means of genetic engineering techniques” of the Federal Office for Consumer Protection and Food Safety (BVL). The working group consists of experts from food and feed inspection authorities of the Federal Länder of Germany, of scientists, and of the involved industry [1]. The guidelines shall contribute to the harmonisation of sampling and testing procedures of food and feed concerning admixtures with unauthorised genetically modified flax in the context of surveillance by the official authorities of the German Federal Länder as well as the in-house control of the industry.

## Sampling of food

Sampling of flax for food purposes is based on the sampling plan for detection of unauthorized GMO developed by the working group of food chemists of the Federal Länder and the BVL (ALS) [2]. This sampling plan is in accordance with European Commission Recommendation 2004/787/EC [3] and in line with the sampling and testing protocol of the Canadian Grain Commission for self-control of Canadian flax shipments to the European Union [4].

By reason of the similar grain weight of the seeds the sample quantity for oilseed rape described in the ALS sampling plan applies accordingly for flax. The sample size for packed or unpacked products is shown in the respective table of the ALS sampling plan [2].

In case of flax four 60 gram sub-samples (each of approx. 10,000 seeds) are taken from the laboratory sample and milled separately. Thus with each sub-sample a detection limit of 0.01% for genetically modified flax is achievable.

## Sampling of feed

The sampling of flax for feed purposes results from a sampling plan for detection of unauthorized GMO that was provided from the VDLUFA working group [5]. This plan is based on the regulation of feed sampling and analysis (FPA) for official feed monitoring and is substantially in accordance with European Commission Recommendation 2004/787/EC [3]. To be consistent with the sampling protocol of the Canadian Grain Commission for self-control of Canadian flax shipments to the European Union [4], four sub-sample analyses each of 60 gram (representing approx. 10,000 seeds) are necessary, i.e. the laboratory sample should consist of at least 240 gram (approx. 40,000 seeds).

## Sample preparation and DNA extraction

One DNA extraction will be made from each milled sub-sample as test portion. The sampling and testing protocol of the Canadian Grain Commission [4] refers to the Fast ID Genomic DNA Extraction Kit [6].

Effective DNA extractions were performed with techniques from the Annexes of ISO 21571 [7] by members of the § 64 working group in particular with the CTAB method (Annex A.3.1), where a minimum sample weight of 1 gram for the DNA extraction step is recommended by the working group.

Other extraction and cleaning methods (kit systems) may be used with less sample weight when indicated, if the comparability is assured.

## PCR analysis

With each sample DNA the PCR analysis will be performed at least as duplicates. The DNA amount per PCR should be approx. 100 ng (photometrically determined).

For the estimated haploid genome weight (1C) of 0.7 pg for flax this amount of DNA corresponds to 140,000 copies of a single-copy target sequence that is present in each of the PCR reactions. For PCR methods with a limit of detection of ten copies a relative detection limit of 0.01% is achievable.

At present, for the detection of genetic modifications in flax (‚CDC Triffid' FP967) the following real-time PCR methods are suitable.

***Detection of T-nos (first screening)***
Real-time PCR based method for amplification of the terminator region DNA sequence of the nopaline synthase gene (T-*nos*) from *Agrobacterium tumefaciens* [8]. The amplified T-nos fragment has a length of 84 bp.

***Detection of P-nos – nptII construct (advanced screening)***
Real-time PCR based method for amplification of the DNA sequence of junction region of a construct consisting of the nopaline synthase gene promoter (P-*nos*) from *Agrobacterium tumefaciens* and the neomycine phosphotransferase II gene (*nptII*) from Tn5 transposon of *Escherichia coli* strain K12 [9]. The length of the amplified fragment depends on the particular GMO and comprises approx. 165 bp for FP967. The method is foreseen to be published in the official collection of analysis methods according to §64 LFGB and was validated in an interlaboratory validation study in November 2009. The procedure of the method is described in detail in annex 1.

***Detection of T-nos - Spec***
Real-time PCR based method for amplification of the DNA sequence of the junction region of the terminator region of the nopaline synthase gene (T-*nos*) from *Agrobacterium tumefaciens* and a sequence region of the dihydrofolate reductase gene from *Escherichia coli*. The length of the amplified fragment is 95 bp. The method was in-house validated and published by the EU Community Reference Laboratory for GM Food and Feed (CRL-GMFF) [10, 11]. The method is suitable for the detection of genetically modified flax event FP967. The method is foreseen to be published in the official collection of analysis methods according to §64 LFGB and was successfully validated in an interlaboratory validation study in November 2009.

***Event-specific detection of FP967***
If the CRL-GMFF or another party provides a validated event-specific real-time PCR technique after publication of this guideline document, this testing method has to be performed according to the instructions described in the user protocol.

***Detection of flax DNA reference gene***
The stearoyl-acyl carrier protein desaturase 2 (SAD) gene can be amplified as reference gene PCR method to test the amplifyability of the sample DNA used the detection of flaxseed DNA. The length of the amplified fragment is 77 bp.

## Interpretation of PCR results

All negative and positive controls shall have the expected results (see ISO 24276 [12]).

If a screening PCR method is used as first PCR analysis step (target sequence *T-nos* or *P-nos-nptII*), the target sequence shall be interpreted as detected, if at least one of the four sample DNAs of the sub-samples is positive in duplicate and shows an increase of the measured fluorescence generated by the amplification.

If the screening result is positive, the sample DNA shall be tested in a further PCR analysis using the T-nos - *Spec* PCR method or (if available) using a FP967 event-specific PCR method.

The genetic modification in flax is detected, if the sample DNA of at least one of the four sub-samples was positive in duplicate in the T-nos - *Spec* PCR or (if available) in a FP967 event specific PCR and shows an increase of the measured fluorescence generated by the amplification.

## References


[1] List of member of the §64 LFGB working group (alphabetical order):
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[2] Probenahmeschema Gentechnik – nicht zugelassene GVO (2008/49) J. Verbr. Lebensm. 3 (2008): 233 – 235. http://www.bvl.bund.de/cln_027/nn_1212624/DE/06__Gentechnik/00__doks__downloads/Probenahmeschema_202008_20engl.html

[3] Commission Recommendation 2004/787/EC of 4 October 2004 on technical guidance for sampling and detection of genetically modified organisms and material produced from genetically modified organisms as or in products in the context of Regulation (EC) No 1830/2003
http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2004:348:0018:0026:EN:PDF

[4] Sampling and Testing Protocol for the Canadian Flaxseed Exported to the European Union. Canadian Grain Commission, Oct. 29 2009; http://www.grainscanada.gc.ca/gmflax-lingm/stpf-peevl-eng.pdf

[5] Probenahme von Futtermitteln zur Untersuchung auf Bestandteile von in der EU zugelassenen GVO im Rahmen einer Überprüfung der Kennzeichnungspflicht. 11/2008. http://www.vdlufa.de

[6] Fast ID Genomic DNA Extraction Kit - Instruction Manual. siehe http://gmo-crl.jrc.ec.europa.eu/flax.htm

[7] ISO 21571:2005, Foodstuffs – Methods of analysis for the detection of genetically modified organisms and derived products – Nucleic acid extraction.

[8] Reiting, R.; Broll, H.; Waiblinger, H. U.; Grohmann, L. (2007) Collaborative study of a T-nos Real-time PCR method for screening of genetically modified organisms in food products. J Verbr Lebensm, 2, 116–121.

[9] Reiting R, unveröffentlicht (siehe Anhang 1)

[10] Report on the Verification of the Performance of a Construct-Specific Assay for the Detection of Flax CDC Triffid Event FP967 Using Real-Time PCR siehe http://gmo-crl.jrc.ec.europa.eu/flax.htm

[11] NOST-Spec construct-specific method for the detection of CDC Triffid Flax (Event FP967) using real-time PCR. siehe http://gmo-crl.jrc.ec.europa.eu/flax.htm

[12] ISO 24276:2006, Foodstuffs – Methods of analysis for the detection of genetically modified organisms and derived products – General requirements and definitions


## Annex 1

Real-time PCR method for the detection of the nopaline synthase promoter (*P-nos*) and neomycin phosphotransferase gene (*nptII*) construct in genetically modified crops.

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