



# Chemisches und Veterinäruntersuchungsamt Freiburg

State Institute for Chemical and Veterinary Analysis, Freiburg

## Detection of genetically modified linseed in food samples - further information

### Sensitivity of the method / DNA extraction

The sensitivity of GMO detection is strongly influenced by the quality of extracted DNA. In case of linseed, it has to be ensured that the influence of inhibitors is minimized. We used the so-called Wizard-preparation (see ISO CEN 21571, Annex A.4) with an additional purification step (gel-filtration columns, e.g. Microspin HR 300, GE Healthcare). The DNA amount used for real-time PCR was estimated to be in the range of 20.000 to 80.000 cp per reaction.

### Sequences detected in screening

Using standardized or commonly used real-time PCR screening methods, positive samples gave almost identical results:

- P35S: negative
- T-nos: positive\*
- pat: negative
- bar: negative
- CTP2-CP4EPSPS-construct: positive\*, caused by botanical impurities (GT 73 rape, see below)
- P-nos: positive\*

\* Threshold cycle 28-33 (T-nos, P-nos) and 35-42 respectively (CTP2-CP4EPSPS) in samples with estimated gm linseed amount of 0,1 to 1percent

### Botanical impurities

Positive screening results (T-nos-sequence, P-nos -sequence) may also be caused by botanic impurities especially of rapeseed, which can often be detected in raw materials of plant origin (e.g. soybeans, mustard).

However, further analyses in linseed samples showed, that - except from CTP2-CP4EPSPS-sequence - positive screening results were not caused by botanic impurities (e.g. rapeseed):



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- DNA of rapeseed and mustard was, if at all, only detectable in very low amounts (CT values in real time PCR higher than 35)
- the possible presence of rapeseed events as MsxXxRfy or Topas 19/2, containing these screening elements p-nos, t-nos and nptII could be excluded by further analyses: the real-time PCR detection of the bar gene and the P35S-sequence respectively was negative.
- Positive findings in CTP2-CP4EPSPS-screening were caused by the presence of Roundup Ready rapeseed (GT 73) in very low trace amounts.

## Detection of gm linseed

For the detection of gm linseed we used construct specific methods, that detect sequence overlaps of different origin being characteristic for plasmid vectors used for genetic transformation. Positive results obtained using construct specific methods give clear indications that gm DNA is present.

For the detection of gm linseed, the combination of two different construct-specific methods was used:

### a) p-nos - nptII

The real-time PCR method was presented to the German network of GMO laboratories by the official laboratory of the land Hessen, LHL. It has been successfully in-house validated in several labs including the CVUA Freiburg, a first interlaboratory study was performed with success and the decision is to standardize the method within § 64 of the German food act in autumn 2009.

The amplification of DNA from gm positive linseed samples resulted in amplicons identical in length and sequence, compared with positive control material of FP967 flax (provided by the official laboratory of Bavaria, the LGL Bayern).

### b) T-nos -Spec

The construct-specific real-time PCR method was developed by Genetic ID, a worldwide operating company specialized in GMO analysis. This method detects a sequence overlap, that is unique for the vector that has been used for transformation of linseed FP967, see also [www.bats.ch/gmo-watch/](http://www.bats.ch/gmo-watch/)

The amplification of DNA from gm positive linseed samples resulted in amplicons identical in length and sequence, compared with positive control material of FP967 flax (provided by the official laboratory of Bavaria, the LGL Bayern).

The method has been made available to the network of GMO laboratories in Europe (ENGL).

## Event-specific method

At the moment there is no event-specific method available, that amplifies DNA from the overlap of linseed specific sequence to sequences of the transformation vector (integration site).

Therefore, at the moment one can only be sure that the sequence characteristic for flax CDC Triffid (FP967) is detectable in the tested samples.

However, a definitive answer, if Event FP 967 is present, can only be given as soon as event-specific methods are available.



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For the assessment of the findings in the context of European Regulation (EC) No. 1829/2003 Article 4 para 2 this question is not relevant, because no gm linseed is authorized at all for marketing as food or feed within the European Union.

## **Estimation of amount of gm-linseed:**

Due to the zero tolerance for non authorized gm-plants in Europe, quantification of gm-amount is not relevant for assessment in context of Article 4 para 2 of European Regulation (EC) No. 1829/2003.

However, estimation of gm-amount is helpful e.g. in order to assess possible influences of sample inhomogeneity on analytical results. Due to the lack of reference material, we used positive control samples as well as a plasmid (supplier of plasmid: Genetic ID Europe).

Using these materials we estimated the amount of gm-linseed in the range of 0,1 to 1 percent for most of the positive samples. In real-time PCR, these amounts correspond to Ct values for both construct specific methods in the range of 28 to 32, indicating a presence of about (at least) 10 to 100 fold higher than the limit of detection.