1 Requirements for extent of GMO testing for raw materials and feed

The minimum requirements for individual enterprises in the non-GMO certification chain are set out in the Non-GMO Standard, Chapter 6.2 - Sample collection and analyses / audit intervals. This document sets out procedures for conducting testing for the presence of GMOs in raw materials and feed.
The information provided in this document **does not take into account all GMO raw materials or additives** approved and tolerated in the EU (e.g. cotton, potatoes, etc.), or that may be included in feed under EU Regulation (EU) 619/2011, including variants of their use. GMOs not permitted in the EU are not part of the minimum testing requirements. In the case of the review of correct labelling of raw materials or feed when other raw materials or additives containing GMOs are used (including other GMOs authorized in the EU, GMOs tolerated in feed under EU Regulation 619/2011 and GMOs not authorized in the EU), the requirements are governed by applicable legislation, particularly Regulations 1829/2003 EC and 1830/2003 EC.

The extent of testing is regularly reviewed, both through audits and consultations of the standard owner with laboratories that carry out GMO testing and, if necessary, the minimum requirements for GMO sample collection and analysis for raw materials and feed are updated. In the case of developments where other GMOs are relevant (e.g. RASFF reports), certified companies will be provided with the appropriate requirements for carrying out analyses on time.

However, the information provided in this document does not imply that certified companies involved in Non-GMO certification are relieved of their obligation to regularly perform their own tests for the presence of GMOs in the raw materials or in the final products (mixtures) and, if necessary, to update the extent of the analyses performed.

1.1 Prevention principle

The principle for preventing contamination or entry of a non-conforming product (GMO-containing raw materials) is testing for the presence of GMOs in the raw materials upon their ***acceptance***. Testing rules allow for the use of primary screening for the presence of GMOs for prevention.
The following can be used for screening*:*

- Indicative FLD method – strip test and indicative method ELISA for uniform commodities such as soybeans, maize, rapeseed, etc..
- PCR screening

See also Section 2.

2 GMO testing - general rules

The following procedure should be followed when testing a sample for the presence of GMOs.
For initial detection, screening for GMOs in the feed or food sample.
All known genetic modifications of a particular raw material must be excluded in the screening. In the event of a positive test result, quantification must be carried out to determine whether the relevant amount is > 0.1% - ≤ 0.9%, > 0.9 or a trace amount of <0.1%, which is considered technically unavoidable.

A generally accepted method is the PCR method for quantification GMOs in a raw material or mixture.
For the purpose of indicative tests, the use of other adequate and validated indicative methods that provide information on the presence of GMOs in a particular single-ingredient or compound feed shall be permitted. If this method is used, it must be approved in advance and an appropriate validation protocol must be submitted.
The PCR method in used for accurate quantification in the accredited laboratory.

Other acceptable methods for testing (screening) and quantification are:

- FLD screening method - strip test and ELISA indicative method for uniform commodities such as soybeans, corn and rapeseed etc. The tests used must be validated for the commodities. In the event of a positive result using the strip test method, quantification by PCR must be performed in an accredited laboratory.
- Or adequate methods with a verification report or verification of accuracy

2.1 Performed testing result approval

The results of the performed analysis must be verified according to the four-eyes principle by an authorized person or another qualified person.

Options of verification with the four-eyes principle:

- In the case of screening using FLD strips, the result must be verified by another person in a specific operation. In the case of a positive test, the test must be performed again - confirmatory test, inspection. This person can be a representative of the supplier, a superior worker in operation, etc.
- When performing the analysis by the PCR method in an accredited laboratory, the verification is performed according to the approved internal quality procedures of the specific accredited laboratory performing the analysis.

2.2 Documentation of performed inspection analyses for the presence of GMOs

2.2.1 Requirements for recording the result of a performed test - FLD strips

The report of the performed testing using FLD strips must contain:

- Unique identification of the sample - name
- Date of sampling, or sample number or other identification (e.g. car license plate, etc.)
- Type - type of analysis performed
- Name of the company or the person having the sample analyzed
- Method of detection
- Date of detection
- Identification of the person who performed the analysis
- Result of the performed analysis with a clear indication of the result - (+ = positive; - = negative); in the event of a positive result of the analysis (+), it is recommended to perform quantification using an accredited PCR method.
- Date of creation of the report
- Identification of the person who has reviewed the result of the analysis performed

A report does not have to be kept if the certified organization performs inspectional indicative analyses of each delivery of raw materials, and keeps a book or other record of performed analyses with clear identification of each specific delivery, sample identification, test result and identification of the person who performed the analysis. Documentation on the performed test must be clearly traceable to the specific delivery and delivery documentation.

2.2.2 Requirements for recording the result of a performed test – PCR method

The report from the accredited laboratory for a performed analysis for the presence of GMOs with the PCR method must comply with the format of a validated method according to the accreditation granted to the laboratory.

2.3 Positive GMO result

In the case of a positive result of the performed analysis - screening, evidence must be available to identify compliance between the organization and the laboratory. For this purpose, the sample must always be uniquely identified. Monitoring and effective measures must be carried out when GMOs that cause positive screening results occur.

Measures include the elimination of impurities (transfers of GMOs from other plant species) from maize, soybean, etc.
In the event of a positive result of an indicative test of a sample of single-ingredient or compound feed carried out according to the sampling plan, the supplier who took the sample and had it tested (dairy farm (SC), Feed mills - producers, distributor, mobile mixer operator, etc.) shall be informed by the laboratory that performed the test.
In the case of an indicative test using a method other than a PCR analysis performed by the laboratory, in the event of a positive result, confirmation and quantification must be performed by the PCR method in an accredited laboratory.

2.4 Follow-up measures in the event of a positive test result

In the event of a positive result of a test for the presence of GMOs in a sample (screening method or PCR method), the supplier or service provider is contacted (Annex 20 – Notifying the supplier of a positive result of the GMO test in feed and supplier's statement).
The occurrence is documented and corrective measures are adopted against the supplier or service provider according to Article 7.1.8 - Remedial measures / continuous improvement of the Non-GMO Standard.
Remedial measures involve confirming the result of the detected GMOs by performing an analysis for the presence of GMOs - quantification by PCR method.

3 Minimum requirements for raw materials / single-ingredient and compound feed

3.1 Minimum requirements for raw materials / single-ingredient feed

3.1.1 Soybean as a raw material or single-ingredient soybean feed

Determining and evaluating the total value of the most important soybean GMOs:

- Quantification GTS 40-3-2 (RRS-1)
- Quantification MON89788 (RRS-2)
- Qualitative detection A2704-12
- Qualitative detection A5547-127 LL

In the case of a positive result for A2704 and A5547, the amount of this GMO can be analyzed by PCR or a similar method that ensures the presence of a sufficient amount of specific DNA. Values higher than < 0.1% must be quantified for a specific amount of GMO content.
The laboratory will work with screening parameters that detect these GMOs.
When identifying GMOs in a raw material or single-ingredient feed, quantification must be carried out and all variants of the GMO must be verified (if the corresponding elements are positive). The GMO presence must be quantified.

The use of the strip test (GMO identification by Strips tests) is permissible for testing, i.e. screening or quantification.
Strip tests can only be applied to specific input commodities and modifications for which the FLD method is validated.
These generally include corn, soybeans, canola, rice and sugar beets.
The use of test strips is only an indicative method for verifying whether GMOs are present in the raw material or in the single-ingredient feed. In the case of positivity, quantification must be performed using the PCR method (see Article 2).

This is a semi-quantitative method for detecting genetic modification of DNA. The method detects modified DNA (screening) with the possibility of quantification using a reader (the software uses approximate quantification.) The test range is <0.1% -> 5%.
The range of the method is:

- < 0.1%
- 0.1-0.5%
- 0.5-1.0%
- 1.0-5.0%
- > 5%

3.1.2 Maize as a raw material or single-ingredient maize feed

1. **Screening for 35S promoter (p35S) and NOS Terminator (tNOS).**

Other screening elements may be also introduced to reduce the corresponding GMO.

2. **If the result is positive: An analysis for at least NK603, TC1507, MON810, MON89034 + RRS-1 must be performed**

If one or more of these parameters have been used in the screening and can be excluded from the specified GMO maize types (not detected), detection of other types of genetic modification must be performed in commercially available GMO maize species under consideration.
In the case of a positive screening result, quantification of the % of GMO content in the sample must be performed.
Exclusion of commercially available types of GMOs in maize must be carried out when testing. If testing has been performed with a negative result, other types of GMOs should also be tested.

3. Quantification of GMOs in maize

Identified GMO types must be quantified when their presence is detected if the concentration is estimated using the PCR method or another similar method, and if the presence of different DNA types exceeding 0.1% (quantification limit) is detected.

RRS-1 positive:

Estimation of soybean content and determining GMO levels in the soybean. It should always be clarified whether it is a relevant amount or a minimum trace amount. If contamination with GMO content is detected, quantification must be performed.

The use of the strip test is permissible for testing, i.e. screening or quantification, and the PCR method must be used for quantification.

Permissible use of the strip test for detection of GMO maize:

- CP4 EPSPS (MON15985XMON88913)
- Cry3Bb1 (MON15985XMON88913)
- Cry1A.105 (MON15985XMON88913)
- Cry1F
- Cry34Ab1
- PAT, MPI
- Cry9C
- VIP3A

Strip tests can only be applied to specific input commodities and modifications for which the FLD method is validated. These generally include corn, soybeans, canola, rice and sugar beets.

The use of test strips is only an indicative method for verifying whether GMOs are present in the raw material or in the single-ingredient feed. In the case of positivity, quantification must be performed using the PCR method (see Article 2).

This is a semi-quantitative method for detecting genetic modification of DNA. The method detects modified DNA (screening) with the possibility of quantification using a reader (the software uses approximate quantification.) The test range is <0.1% -> 5%.

The range of the method is:

- < 0.1%
- 0.1-0.5%
- 0.5-1.0%
- 1.0-5.0%
- > 5%

3.1.3 Minimum requirements for rapeseed as a raw material or single-ingredient rapeseed feed

1. Triple screening

Detection of all relevant GMO varieties of rapeseed (e.g. TNOS, PAT gene (or LibertyLink construct), CTP2-CP4psps (or pFMV))

2. ID depending on positive screening results

- tNOS positive: at least RRS + bar gen for MS8 / RF3 or both
- PAT gen / LibertyLink positive: at least rapeseed T45
- CTP2-CP4epsps / pFMV positive: at least GT73

3. Quantification of GMO rapeseed

The identified varieties of genetically modified rapeseed must be quantified if the PCR method or another method that ensures the presence of sufficient DNA yields values above the limit of 0.1% is used for the quantitative estimation.

Positive screening results must be clarified.

If no GMO rapeseed is detected, it must be verified that there is no botanical contamination containing GMO soybean or maize (mass estimation and evaluation). Is it a relevant amount or a trace amount? If GMO contamination is detected, an official analysis must be performed according to a verified methodology (PCR).

3.2 Minimum requirements for compound feed

3.2.1 Minimum requirements for compound feed containing soybean

Determining and evaluating quantification of the most important GMOs:
Soybean:

- Quantification GTS 40-3-2 (RRS-1)
- Quantification MON89788 (RRS-2)
- Qualitative detection A2704-12
- Qualitative detection A5547-127 LL

In the case of a positive result for A2704 and A5547, the amount of this GMO can be analyzed by the PCR or a similar method to ensure that a sufficient amount of the DNA type is present (e.g. total soybean content in the sample). Values greater than 0.1% must be quantified to determine whether the amount of GMOs is in the range of > 0.1% - ≤ 0.9% or> 0.9%.
In the case of limited analysis of the soybean component, the practical LOD (limit of detection) must be reported.

For maize component:
Other qualitative detection of 4 commercially available modified maize varieties: NK603, TC1507, MON810, MON89034.

In the event of a positive result, the amount of this GMO can be analyzed by PCR or a similar method to ensure that a specific DNA type is present. Values greater than 0.1% must be quantified for detected GMOs.

In the case of limited analyzability of the maize component, the practical LOD (limit of detection) must be reported.

For rapeseed components:
Other qualitative detection of rapeseed in compound feed are GT73, MS8, RF3 (bar Gen modification.

In the case of positive identification of GT73, MS8, RF3, quantification and quantity estimation, if necessary, must be carried out by PCR or another similar method to determine whether a specific amount of DNA type is present leading to values above 0.1%.

In the case of limited analyzability of the rapeseed component, the practical LOD must be reported.

Alternatively, the laboratory may work with screening parameters that detect at least the specified GMOs (soybean, rapeseed, maize). In subsequent identification / quantification of positive results, all GMOs must be verified (if the relevant elements are positive). All the listed GMOs must be identified and quantified, if necessary, with a PCR analysis.

3.2.2 Minimum requirements for compound feed containing soybean, maize and rapeseed seeds

Determining and quantifying the most important GMOs:
Soybean mass estimation:
The first step is to estimate the amount of soybean in the feed. For quantities greater than 0.9% of GMO soybean, an assessment must be determined (see minimum requirements for feed containing soybean) and performed according to the official directive1.

For component from rapeseed seed:
Qualitative evidence of GT73 seed + MS8 seed or RF3 seed (or bar Gene)

In the case of positive identification, quantification and detection of GMOs or multiple GMO species must be performed with quantity estimation using PCR or another similar method to ensure that it is sufficient. If DNA types are present in amounts leading to values above 0.1%.

In the case of limited analyzability of the maize component, the practical LOD must be reported.

For maize component:

Qualitative evidence of 4 commercially used maize varieties: NK603, TC1507, MON810, MON89034.

In the case of positive identification, quantification and detection of GMOs or multiple GMO species must be performed with quantity estimation using PCR or another similar method to ensure that it is sufficient. If DNA types are present in amounts leading to values above 0.1%.

In the case of limited analyzability of the maize component, the practical LOD (limit of detection) must be reported.

Alternatively, the laboratory may work with screening parameters that detect at least the specified GMOs (soybean, rapeseed, maize). In subsequent identification / quantification of positive results, all GMOs (if the relevant elements are positive) listed here must be verified and identified, as well as quantified, if necessary.

4 Other products / raw materials

Strategies for the analysis of GMOs in other single-ingredient feed, raw materials, (food) components, intermediate products or food shall continue to be approved with the designated laboratories with regard to the composition and origin of the products/components. Strip tests can only be applied to specific input commodities and modifications for which the FLD screening method is validated; for rice mostly PAT - Liberty Link construct LLRice 62, LLRice61, for cotton mostly Cry1Ac, DMO. In the event of a positive test result, PCR quantification must be performed.
For other raw materials, the PCR or another similar method is recommended.