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BIOTECON Diagnostics

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GMOs

GMO Regulations

Since the beginning of the commercial use of genetically modified plants more than 20 years ago, over 350 different transgenic plants have been introduced into the market. These genetically modified organisms (GMOs) with novel traits are resistant to herbicides, stress, pests or viruses, have a longer shelf life, additional nutritional benefits, or possess enhanced production of raw materials like oil or starch. Unlike other genetic improvement methods, the application of this technology is strictly regulated in many countries.

Global GMO Regulations

Over 40 countries have implemented legislation for the use of GMOs regarding their cultivation and labeling of food containing them. There is large variation among these regulations. Some require a zero tolerance against GMOs, while other regulations allow their use under certain conditions. In some countries the product must be labeled if containing GMOs, when a defined threshold is crossed, while in other countries labeling is voluntary. Different regulations need different approaches for detection, identification and quantification of GMO content in food and feed.

Overview of GMO Regulations in the European Union

The European Union has established a legal framework to strictly regulate the use of GMOs. The main regulations are Directive 2001/18/EC for the deliberate release of GMOs into the environment, Regulation (EC) No 1829/2003 for placing GMO food and feed into the market and (EC) No 1830/2003 for GMO labelling and traceability requirements amending Directive 2001/18/EC. Additionally, Directive (EU) 2015/412 allows EU Member States to restrict or prohibit the cultivation of GMOs in their territory.

European Union Regulations in Brief

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- Zero tolerance for unauthorized GMOs: Only authorized GMOs may be used in food and feed; unauthorized GMO events are forbidden and traces of them are not accepted.
- Labeling: Authorized GMO events may be used, but respective products must be labeled.
- Threshold: Unintended and unavoidable contamination with authorized GMO events need not be labeled if they are below a threshold of 0.9% GMO content in the respective ingredient.
- Additional regulations on a national level are possible, e.g. the cultivation of MON810 is banned in some EU countries.

For reliable and cost-effective detection of GMOs in food and feed samples, BIOTECON Diagnostics offers a practical, comprehensive GMO testing strategy including screening, identification and quantification of GMOs in accordance with EU regulations. This brochure provides an overview of BIOTECON Diagnostics' GMO testing strategy.

How are GM plants designed?

For a better understanding of how to detect and identify a specific genetically modified plant, it is useful to know how a GMO is made. To provide new abilities to a plant, a novel gene sequence is integrated into the plant´s genome. The integrated DNA “construct” contains all information needed to express the new desired characteristics. Often it consists of regularly used genetic elements from the molecular biology “toolbox”. For example, a construct contains a promoter for initiation of gene transcription, the gene of interest carrying the new ability (e.g. herbicide resistance), a terminator which signals the termination of gene transcription and a marker which helps select successfully transformed plants (Fig. 1). The successful integration of an artificial genetic construct into the plant genome, giving rise to a transgenic plant, is called an “event”.

Real-time PCR is the most reliable method to detect GMOs in food and feed samples, because its target is this genetic information. Moreover, DNA is a very stable molecule, and real-time PCR is highly specific, sensitive and time-saving. Multiplex real-time PCR facilitates the simultaneous detection and differentiation of several gene targets within one real-time PCR reaction tube, making it perfect for screening. Real-time PCR can be reliably used for identification and differentiation of events and for quantification of the relative GMO content.

Questions for GM Food and Feed Analysis

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1. DOES A SAMPLE CONTAIN GMO?
   Can be answered by **screening**
2. WHICH EVENTS ARE IN THE SAMPLE? ARE THESE EVENTS AUTHORIZED?
   Can be answered by **identification**
3. IF IT IS AUTHORIZED, IS THE RELATIVE CONTENT ABOVE LEGAL LIMITS?
   Can be answered by **quantification**

Frequently used “toolbox” elements are perfect target sequences for screening. The transition sequence between the original plant DNA and the introduced construct is event specific and can be used for identification or in context with quantification (Fig. 1).

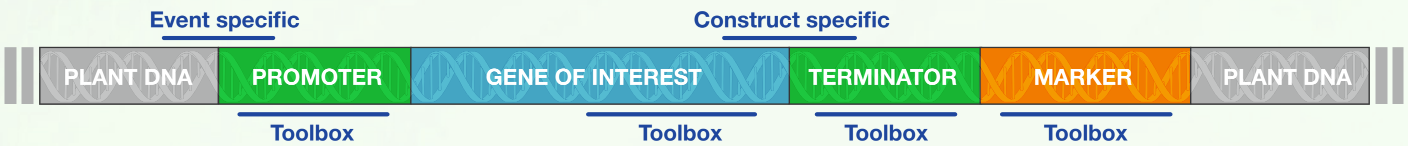

Figure 1. Integration of a novel DNA sequence, flanked by functional elements, into the plant´s genome. Toolbox sequences which can be used for screening are marked.

GMO Screening Strategy

Many genetically modified plants share commonly used genetic "toolbox" elements, independent from plant type, such as maize, soya, cotton or canola. These can be regulatory sequences like promoters and terminators, marker genes or widely used active genes like insect or herbicide resistance (Fig. 1). Since there is no sequence which is present in all GMO events, a combination of targets is used to cover as many events as possible (Table 1).

In the past, the combined detection of the 35S promoter and NOS terminator provided good certainty of the presence or absence of GMOs in a sample. However, the development of new GMO events not containing P-35S or T-NOS, e.g. Roundup Ready 2 Yield soya (MON89788) or H7 sugar beet, made this approach insufficient.

BIOTECON Diagnostics' GMO screening strategy consists of a sophisticated combination of screening assays: The **food**proof® GMO Screening 1 LyoKit and the **food**proof® GMO Screening 2 LyoKit contain 8 targets in total to receive the most information with the least amount of effort for maximum coverage about the presence of GMOs.

Regulatory Elements

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- **P-35S:** 35S Promoter, a strong, constitutive promoter derived from the cauliflower mosaic virus (CaMV).
- **T-NOS:** Terminator sequence derived from *Agrobacterium tumefaciens*. In this organism, this sequence terminates the transcription of the nopalin synthetase (nos) gene, which is injected into a plant cell and activated there.
- ***bar***: The bar resistance gene (phosphinothricin N-acetyl-transferase) transfers herbicide resistance and originates from the soil bacterium *Streptomyces hygroscopicus*.
- **P-FMV**: Similar to P-35S, it is a strong constitutive promoter derived from the figwort mosaic virus (FMV).
- **CTP2-CP4-EPSPS:** A construct out of the chloroplast targeting sequence from *Arabidopsis thaliana* EPSPS and the EPSPS gene isolated from *Agrobacterium* sp. strain CP4.

The **food**proof® Plant Detection LyoKit detects plant DNA in raw material, processed food, feed and seed samples. The risk of false-negative results caused by degraded DNA can be eliminated using this kit. Additionally, the assay can be used as a process control.

Table 1. Available lyophilized GMO screening kits

Screening Assay	Targets	Limit of Detection
foodproof® GMO Screening 1 LyoKit	P-35S, T-NOS, P-FMV, Internal Control	0.01%
foodproof® GMO Screening 2 LyoKit	bar, P-35S-pat, CTP2-CP4-EPSPS, P-NOS-nptII, P-35S-nptII	0.01%
foodproof® Plant Detection LyoKit	Plant, Internal Control	0.25 target copies/reactions

GMO Event Identification

Figure 2. GMO screening workflow

Every screening approach, no matter how well-thought-out, leaves a small gap. GM plants, which do not contain one of the targeted sequences, remain undetected. For instance, events like MON87708, BPS-CV127-9 or MON87769 do not share commonly used “toolbox” elements.

To close this gap, BIOTECON Diagnostics designed a series of multiplex identification kits: The **food**proof® GMO Soya Identification 1 LyoKit and **food**proof® GMO Soya Identification 2 LyoKit, which identify four soya events each in one multiplex reaction, respectively, and the **food**proof® GMO Maize Identification 1 LyoKit, which detects three maize events not covered by screening. The result is a direct identification of the targeted events. In combination with BIOTECON Diagnostics screening assays, a comprehensive detection and secure analysis of soya and maize events can be done.

Table 2. Available multiplex GMO event identification kits

Identification Assay	Event
foodproof® GMO Soya Identification 1 LyoKit	DAS-44406-6, MON87701, MON87708, MON87769
foodproof® GMO Soya Identification 2 LyoKit	BPS-CV127-9, DP-305423-1, DAS-68416-4, DAS-81419-2
foodproof® GMO Maize Identification 1 LyoKit	DAS-40278-9, LY038, VCO-01981-5

Screening Targets

*Table 3. Overview of GMO events detected by **food**proof kits*

Plant	GMO Event	**food**proof **Quantification Kits**	**food**proof **SL Kits** ((M) = Multiplex Kits)	GMO Screening 2 LyoKit: **P-35S-nptII** [Cy5]	GMO Screening 2 LyoKit: **P-NOS-nptII** [Cy5]	GMO Screening 2 LyoKit: **CTP2-CP4EPSPS** [ROX]	GMO Screening 2 LyoKit: **P-35S-pat** [HEX]	GMO Screening 2 LyoKit: **bar** [FAM]	GMO Screening 1 LyoKit: **P-FMV** [ROX]	GMO Screening 1 LyoKit: **T-NOS** [HEX]	GMO Screening 1 LyoKit: **P-35S** [FAM]
Soya (*Glycine max*)	DP356043 (GAT, Optimum)			-	-	-	-	-	-	-	+
	A2704-12 (Liberty Link)		Z 722 01	-	-	-	+	-	-	-	+
	A2704-21, A5547-35 (Liberty Link)			-	-	-	+	-	-	-	+
	A5547-127 (Liberty Link)			-	-	-	+	-	-	-	+
	GU262 (Liberty Link)			-	-	-	+	-	-	-	+
	FG72 (Balance)			-	-	-	-	-	-	+	-
	G94-1, G94-19, G-168 (Optimum)			-	-	-	-	-	-	+	+
	GTS 40-3-2 (Roundup Ready)	R 300 19 R 302 19	Z 722 02	-	-	-	-	-	-	+	+
	260-05 (G94-1, G-168, G94-19)			-	-	-	-	-	-	+	+
	SYHT0H2			-	-	-	+	-	-	+	+
	W62, W98 (Liberty Link)			-	-	-	-	+	-	+	+
	MON87705 (Vistive Gold)			-	-	+	-	-	+	-	-
	MON89788 (Genuity Roundup Ready 2 Yield)	R 302 35		-	-	+	-	-	+	-	-
Maize (*Zea mays*)	Event 98140 (Optimum GAT)	R 302 29		-	-	-	-	-	-	-	+
	MON810 (YieldGard Corn Borer)	R 302 29	Z 725 07 (M)	-	-	-	-	-	-	-	+
	MON87427 (Roundup Ready)	R 302 29		-	-	-	-	-	-	-	+
	B16 (DLL25)	R 302 29		-	-	-	-	+	-	-	+
	Bt176 (NaturGard KnockOut, Maximizer)	R 302 29 R 302 16	Z 725 02 (M)	-	-	-	-	+	-	-	+
	DAS-06275-8	R 302 29		-	-	-	-	+	-	-	+
	DBT418 (Bt-Xtra)	R 302 29		-	-	-	-	+	-	-	+
	TC-6275	R 302 29		-	-	-	-	+	-	-	+
	DAS-59132-8 (Event 32, E-32)	R 302 29		-	-	-	+	-	-	-	+
	4114	R 302 29		-	-	-	+	-	-	-	+
	676, 678, 680	R 302 29		-	-	-	+	-	-	-	+
	DAS-59122-7 (Herculex RW)	R 302 29	Z 720 11	-	-	-	+	-	-	-	+
	DAS-1507 (TC1507, Herculex)	R 302 29	Z 725 06 (M)	-	-	-	+	-	-	-	+
	T14	R 302 29		-	-	-	+	-	-	-	+
	T25	R 302 29	Z 725 07 (M)	-	-	-	+	-	-	-	+
	Event 3272 (Enogen)			-	-	-	-	-	-	+	-
	GA21 (Roundup Ready)		Z 725 01 (M)	-	-	-	-	-	-	+	-
	MIR604 (Agrisure RW)		Z 725 01 (M)	-	-	-	-	-	-	+	-
	MIR162 (Agrisure Viptera)		Z 725 03 (M)	-	-	-	-	-	-	+	-
	5307 (Duracade)			-	-	-	-	-	-	+	-
	MON863 (YieldGard)	R 302 29	Z 725 07 (M)	+	-	-	-	-	-	+	+
	MON87460 (Genuity DroughtGard)	R 302 29		+	-	-	-	-	-	+	+
	CBH-351 (StarLink)	R 302 29	Z 725 02 (M)	-	-	-	-	+	-	+	+
	MS3 (SeedLink)	R 302 29		-	-	-	-	+	-	+	+
	MS6 (SeedLink)	R 302 29		-	-	-	-	+	-	+	+
	MON802	R 302 29		+	-	+	-	-	-	+	+
	MON832 (Roundup Ready)	R 302 29		+	-	+	-	-	-	+	+
	MON80100	R 302 29		+	-	+	-	-	-	+	+
	MON809 (YieldGard)	R 302 29		-	-	+	-	-	-	+	+
	MON88017 (YieldGard VT Rootworm/RR2)	R 302 29	Z 725 03 (M)	-	-	+	-	-	-	+	+
	NK 603 (Roundup Ready)	R 302 29	Z 725 03 (M)	-	-	+	-	-	-	+	+
	Bt11 (Agrisure CB/LL)	R 302 29	Z 725 06 (M)	-	-	-	(+)*	-	-	+	+
	MON89034 (YieldGard VT Pro)	R 302 29	Z 725 02 (M)	-	-	-	-	-	+	+	+
Canola (*Brassica napus*)	Laurical 23-198 (Event 23)			+	-	-	-	-	-	-	+
	Laurical 23-18-17 (Event 18)			+	-	-	-	-	-	-	+
	Falcon GS40/90			-	-	-	+	-	-	-	+
	Liberator L62 (pHoe6/AC)			-	-	-	+	-	-	-	+
	Topas19/2 (HCN 10, HCN 92 LibertyLink) (AOCS Ref.)			-	+	-	+	-	-	-	+
	T45, HCN 28 (LibertyLink)			-	-	-	+	-	-	-	+
	Ms1 (InVigor)			-	+	-	-	+	-	+	-
	Ms1xRf1 (PGS1) (InVigor)			-	+	-	-	+	-	+	-
	Ms1xRf2 (PGS2) (InVigor)			-	+	-	-	+	-	+	-
	RF1, RF2			-	+	-	-	+	-	+	-
	RF3			-	-	-	-	+	-	+	-

*detected at very low sensitivity because of shorter fragment

Plant	GMO Event	foodproof Quantification Kits	foodproof SL Kits (M) = Multiplex Kits	GMO Screening 2 LyoKit: P-35S-nptII [Cy5]	GMO Screening 2 LyoKit: P-NOS-nptII [Cy5]	GMO Screening 2 LyoKit: CTP2-CP4EPSPS [ROX]	GMO Screening 2 LyoKit: P-35S-pat [HEX]	GMO Screening 2 LyoKit: bar [FAM]	GMO Screening 1 LyoKit: P-FMV [ROX]	GMO Screening 1 LyoKit: T-NOS [HEX]	GMO Screening 1 LyoKit: P-35S [FAM]
	MS8			-	-	-	-	+	-	+	-
	MS8xRF3			-	-	-	-	+	-	+	-
	GT73, GT200			-	-	+	-	-	+	-	-
	MON88302			-	-	+	-	-	+	-	-
	OXY 235			-	-	-	-	-	-	+	+
	MPS965			-	-	-	-	-	-	+	-
	ZSR500, ZSR502, ZSR503			-	-	-	-	-	+	-	-
	PHY14, PHY23, PHY35, PHY36			-	-	-	-	+	-	-	-
Rice (*Oryza sativa*)	LLRICE62 (LL62, LibertyLink)			-	-	-	-	+	-	-	+
Rice (*Oryza sativa*)	LLRICE06 (LL06)			-	-	-	-	+	-	-	+
Rice (*Oryza sativa*)	LLRICE601 (LibertyLink), LLRICE604			-	-	-	-	+	-	-	+
Rice (*Oryza sativa*)	T1c-19 Rice			-	-	-	-	+	-	-	+
Rice (*Oryza sativa*)	Bt-ZJ22			-	-	-	-	-	-	+	-
Rice (*Oryza sativa*)	Bt63 (TT51-1)			-	-	-	-	-	-	+	-
Rice (*Oryza sativa*)	GR2 Rice (Golden Rice 2)			-	-	-	-	-	-	+	-
Rice (*Oryza sativa*)	KeFeng6			-	-	-	-	-	-	+	+
Rice (*Oryza sativa*)	Kemingdao1 (KMD1)			-	-	-	-	-	-	+	+
Potato (Solanum tuberosum)	SPBT02-5			+	-	-	-	-	-	-	+
Potato (Solanum tuberosum)	ATBT04-X (New Leaf)			+	-	-	-	-	-	+	+
Potato (Solanum tuberosum)	SPBT02-7			+	-	-	-	-	-	+	+
Potato (Solanum tuberosum)	BT6, BT10, Bt12, BT16, BT17, BT18, BT23 (New Leaf)			+	-	-	-	-	-	+	+
Potato (Solanum tuberosum)	PH05 - 026 - 0048 (Fortuna)			-	-	-	-	-	-	+	-
Potato (Solanum tuberosum)	AM 04-1020 (Amadea)			-	-	-	-	-	-	+	-
Potato (Solanum tuberosum)	AV43-6-G7			-	-	-	-	-	-	+	-
Potato (Solanum tuberosum)	EH92-527-1			-	+	-	-	-	-	+	-
Potato (Solanum tuberosum)	RBMT21-129, RBMT21-350 (New Leaf)			-	+	-	-	-	+	+	-
Potato (Solanum tuberosum)	RBMT21-152 (New Leaf)			-	-	-	-	-	+	+	-
Potato (Solanum tuberosum)	RBMT22-082, RBMT22-186, RBMT22-238, RBMT22-262 (New Leaf)			-	-	+	-	-	+	+	-
Potato (Solanum tuberosum)	SEMT15-15, HLMT15-46 (New Leaf)			-	-	-	-	-	+	+	-
Potato (Solanum tuberosum)	RBMT15-101, SEMT15-02			+	-	-	-	-	+	+	+
Tomato (*Solanum lycopersicum*)	CGN-89564-2 FlavrSavr			-	-	-	-	-	-	-	+
Tomato (*Solanum lycopersicum*)	35 1 N			-	+	-	-	-	-	+	-
Tomato (*Solanum lycopersicum*)	1345-4 (Endless summer)			-	+	-	-	-	-	+	+
Tomato (*Solanum lycopersicum*)	B, Da. F (Vegadura)			-	+	-	-	-	-	+	+
Tomato (*Solanum lycopersicum*)	5345			+	-	-	-	-	-	+	+
Tomato (*Solanum lycopersicum*)	Huafan No 1			-	-	-	-	-	-	+	+
Tomato (*Solanum lycopersicum*)	8338			+	-	-	-	-	+	+	+
Sugar Beet (*Beta vulgaris*)	T120-7 (LibertyLink)			-	+	-	+	-	-	-	+
Sugar Beet (*Beta vulgaris*)	GTSB77 (Roundup Ready)			-	-	+	-	-	+	-	+
Sugar Beet (*Beta vulgaris*)	H7-1 (Roundup Ready)			-	-	+	-	-	+	-	-
Sugar Beet (*Beta vulgaris*)	SBVR111			-	-	-	-	-	-	+	-
Cotton (*Gossypium hirsutum*)	31807, 31808			+	-	-	-	-	-	-	+
Cotton (*Gossypium hirsutum*)	BXN10211			-	-	-	-	-	-	-	+
Cotton (*Gossypium hirsutum*)	COT67B, COT102			-	-	-	-	-	-	+	-
Cotton (*Gossypium hirsutum*)	MON531 (Bollgard)			+	-	-	-	-	-	+	+
Cotton (*Gossypium hirsutum*)	Event 1 (JK 1)			+	-	-	-	-	-	+	+
Cotton (*Gossypium hirsutum*)	MON757			+	-	-	-	-	-	+	+
Cotton (*Gossypium hirsutum*)	MON15985 (Bollgard II)			+	-	-	-	-	-	+	+
Cotton (*Gossypium hirsutum*)	MON1445 (Roundup Ready)			+	-	+	-	-	-	+	+
Cotton (*Gossypium hirsutum*)	T304-40			-	-	-	-	+	-	+	+
Cotton (*Gossypium hirsutum*)	GHB119			-	-	-	-	+	-	+	+
Cotton (*Gossypium hirsutum*)	LLCotton25 (LibertyLink)			-	-	-	-	+	-	+	+
Cotton (*Gossypium hirsutum*)	MON88701			-	-	-	-	+	-	+	+
Cotton (*Gossypium hirsutum*)	MON88913 (Genuity Roundup Ready Flex)			-	-	+	-	-	+	-	+
Cotton (*Gossypium hirsutum*)	MON1076			+	-	-	-	-	+	+	+
Cotton (*Gossypium hirsutum*)	MON1698			+	-	-	-	-	+	+	+

GMO Event Identification

Following screening, the acquired information can directly be used for further analysis. Still it may be necessary to perform many tests to identify all of the GMOs inside the sample, if screening results turn out positive. With a sophisticated strategy using multiplexing, effort, cost and time can be minimized.

The **food**proof® SL GMO Maize Multiplex Identification Kits facilitate a fast, safe and easy qualitative detection of GM ingredients in food and feed. Additionally, BIOTECON Diagnostics **food**proof® SL GMO Identification Kits cover the most commonly found soya and maize events. In the European Union, identified events are classified as authorized or unauthorized. Unauthorized GMOs are not allowed in the EU. The relative content of authorized events inside the sample must be quantified for labeling.

International ISO Standards for Food and Feed

Methods of analysis for the detection of genetically modified organisms and derived products:

- **ISO 24276:** General requirements and definitions.
- **EN/TS 21568:** Sampling strategies.
- **ISO 21569:** Qualitative nucleic acid based methods.
- **ISO 21570:** Quantitative nucleic acid based methods.
- **ISO 21571:** Nucleic acid extraction.

BIOTECON Diagnostics GMO Kits and LyoKits were developed according to the specifications of ISO Methods and the German Food Law § 64 LFGB.

Table 4. Recommended certified reference material for validation

Name of Event	Plant	GMO Screening 1			GMO Screening 2				Certified Reference Material
		P-35S	T-NOS	FMV-35S	bar	35S-pat	CTP2-CP4EPSPS	P-NOS-nptII P-35S-nptII	
EH92-527-1	potato	-	+	-	-	-	-	+/-	ERM-BF421B; (100% GMO)
Bt176	maize	+	-	-	+	-	-	-	ERM-BF411B; (0.1% GMO)
59122	maize	+	-	-	-	+	-	-	ERM-BF424B; (0.1% GMO)
NK 603	maize	+	+	-	-	-	+	-	ERM-BF415B; (0.1% GMO)
GTS 40-3-2	soya	+	+	-	-	-	-	-	ERM-BF410BK; (0.1% GMO)
MON 89788	soya	-	-	+	-	-	+	-	AOCS 0906-B; (100% GMO)

Note: Unexpected positive results with Certified Reference Materials (CRMs) can occur, if traces of another GMO are present and are detected. CRMs are verified for the presence of a specific GM event but not for the absence of other possibly contaminating GM events. (CEN/TS 16707)

GMO Quantification

In many countries, labeling of food and feed products containing GMOs is required, and a threshold for GMO content in products or ingredients is defined. In the European Union, the presence of authorized GMOs in a product without labeling is allowed if the contamination with GMO was unavoidable, unintended and does not exceed 0.9% relative GMO content. GMOs that are authorized elsewhere and are in the EU authorization process may be present in feed with up to 0.1% of the ingredient. Regulations like these require specific methods to reliably and correctly quantify GMOs. GMO quantification always determines the relative GMO content: the ratio between the total copy number of the GMO-specific target (e.g. Roundup Ready 2 Yield soybean) and the total copy number of the plant species target (e.g. soybean), which is calculated and expressed as a percentage (% GMO soya of total soya content). The copy number is calculated with the help of respective standard curves, which are measured in parallel (Fig. 4). BIOTECON Diagnostics offers the **food**proof® GMO Quantification Kits for reliable quantification of the most important events, e.g. Roundup Ready Soya and Roundup Ready 2 Yield Soya. The **food**proof® GMO 35S Maize Quantification Kit facilitates quantification of all GMO maize events containing the 35S promoter. The **food**proof® GMO Quantification Kits contain all necessary components for relative quantification, including calibrator DNA for standard curves.

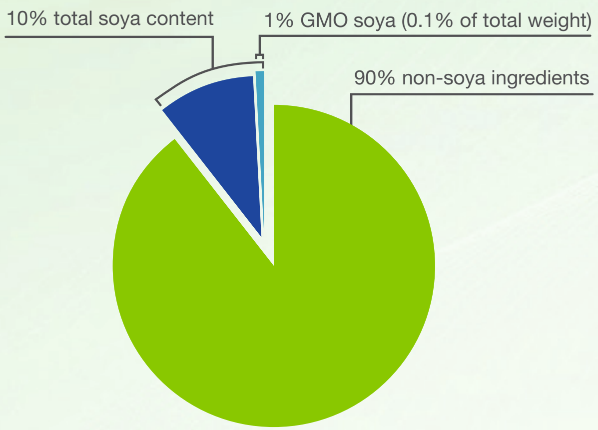
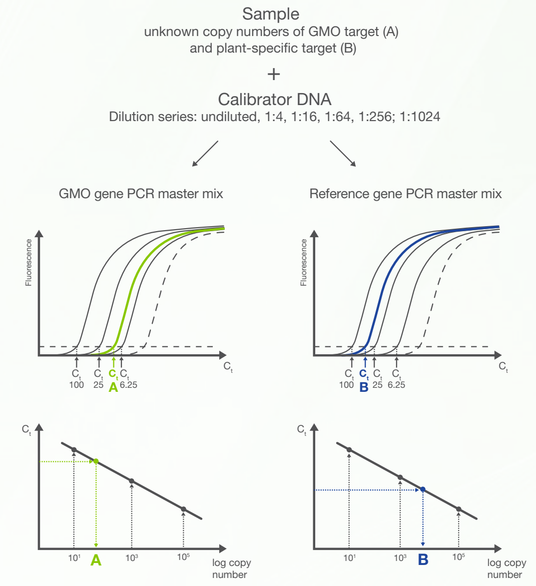


Figure 3. The difference between absolute and relative GMO content

Official methods and regulations may require the determination of the relative GMO content. In European regulations, the GMO content is defined as the percentage of GMO DNA copy number in relation to plant specific DNA copy number, calculated in terms of haploid genomes. An absolute quantification would be cumbersome and cost-intensive, and therefore is not reasonable for a routine service laboratory.

Relative GMO Quantification



Relative GMO Content [%]:

$$\frac{A \text{ (copy number of GMO target)}}{B \text{ (copy number of plant-specific target)}} \times 100 / N$$

N: normalization factor

Figure 4. Relative GMO Quantification

The Calibrator DNA and Dilution Buffer to create the standard curves are provided with the our kits. Additionally, the Calibrator DNA serves as a positive control and as a reference to normalize the relative DNA copy number ratio. Normalization corrects the differences resulting from the combined variation in the quantity and quality of DNA samples and the efficiency of the real-time PCR.

Manual DNA Extraction

The first step in GMO analysis is sampling. Samples should be taken according to a defined procedure to obtain a representative sample of the matrix being tested. Respective sampling strategies can be found in official methods and regulations, such as EN/TS 21568 or EU Regulation No 691/2013. BIOTECON Diagnostics supports you in developing optimized sampling plans and offers a broad range of laboratory equipment for homogenization, mixing and cell disruption.

DNA Extraction

Following sample homogenization, the DNA must be extracted from the plant cells. As plant material can contain polysaccharides, phenols, and other potential PCR inhibitors, proper GMO detection requires good isolation and clean-up of the DNA. Furthermore, if a sample matrix is high in protein content, this must be removed before DNA extraction.

With the **food**proof® Sample Preparation Kit III, BIOTECON Diagnostics offers an innovative, quick, and user-friendly alternative to conventional DNA isolation methods, which are tedious and require the use of highly toxic chemicals. Whether the product is a raw commodity, an intermediate material or a highly processed product, optimized protocols support a comfortable and effective lysis of plant cells as well as purification of DNA using spin columns.

The cell walls are disrupted at high temperature with the provided extraction buffer. After centrifugation and proteinase K digestion, the DNA is bound selectively to special fibers in the spin columns. The bound DNA is purified in two "wash- and -spin" steps and finally released from the fibers with a low salt elution.

*Figure 5. **food**proof® Sample Preparation Kit III*

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Kit Features

- **Optimized reagents and protocols** for raw materials and foodstuffs
- Purification of DNA from **plant or animal** materials
- **Simple and easy** protocol
- Isolation of **highly purified DNA**, free from other cellular components and PCR inhibitors
- **Validated** combination of sample preparation and PCR detection

Automated DNA Extraction

Automation of DNA extraction is key to higher efficiency and reproducibility in a routine lab. BIOTECON Diagnostics offers a complete solution for this approach: the **food**proof® Magnetic Preparation Kit III in combination with the KingFisher™ Flex instrument.

GMO Automation: DNA Extraction Kit

The **food**proof® Magnetic Preparation Kit III is designed for automated DNA extraction from food samples using magnetic beads. The beads are incubated with the lysed sample and bind the released DNA. Washing steps eliminate unwanted inhibitors.

Due to its innovative technology highly purifed DNA is isolated even from complex matrices and used for qualitative and quantitative testing of GMOs.

GMO Automation: Robot

The extraction is performed very quickly and safely on the KingFisher™ Flex. The unique feature of transfering the magnetic beads with the bound DNA rather than liquid provides a higher yield of pure DNA. The robot processes up to 96 samples at once in only 35 minutes with no risk of cross-contamination. The system was validated for laboratories and food manufacturers with medium to high sample throughput. Additionally this system runs with other Magnetic Preparation Kits from BIOTECON Diagnostics for pathogen, allergen and animal identification analysis.

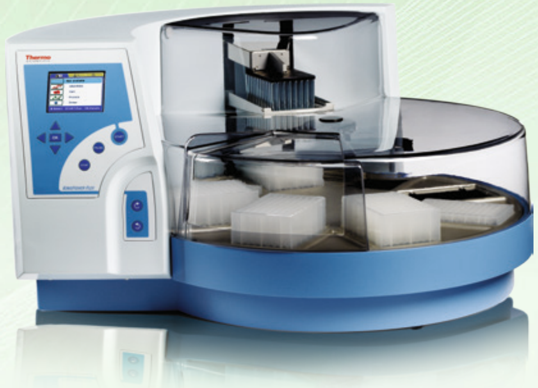


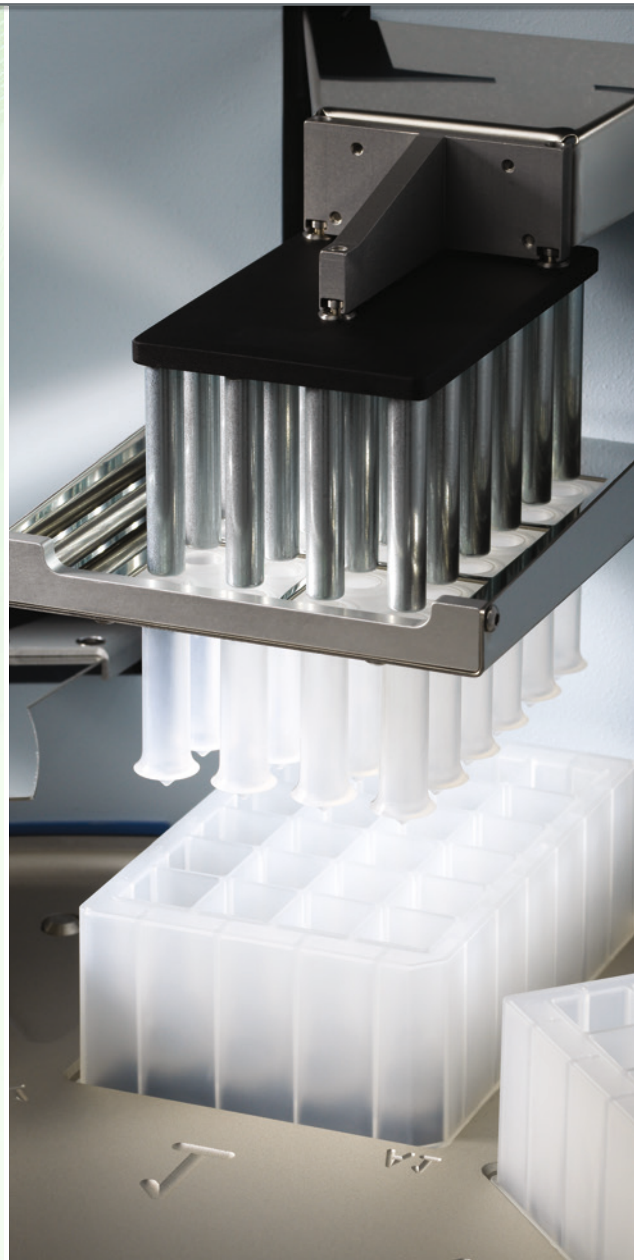
Figure 6. KingFisher™ Flex for automated DNA extraction

The KingFisher™ Flex Technology

- Transfers magnetic beads with bound DNA through the different washing steps, not the liquid
- During elution the purified DNA is released to the liquid and is ready for analysis
- Exchangeable magnetic head with 24 or 96 pins

Benefits

- High yield of pure DNA
- Very fast purification (35 min) even with up to 96 samples
- Up to 1 g sample can be analyzed
- Easy-to-use control software
- Open for a variety of applications: GMO, pathogen, allergen and animal identification analysis



Product List

GMO Screening Kits

R 300 17	**food**proof® GMO Screening Kit (35S, NOS), plant assay included - LC 1.x, 2.0, 480 -
R 302 17	**food**proof® GMO Screening Kit (35S, NOS, bar, FMV), plant assay included
R 602 17	**food**proof® GMO Screening 1 LyoKit (35S, NOS, FMV)
R 602 18	**food**proof® GMO Screening 2 LyoKit (bar, P-35S-pat, CTP2-CP4-EPSPS, P-NOS-nptII, P-35S-nptII)
R 602 21	**food**proof® Plant Detection LyoKit
RDK 302 39	**food**proof® CaMV Detection Kit

GMO Identification Kits*

R 602 24	**food**proof® GMO Soya Identification 1 LyoKit (DAS-44406-6, MON87701, MON87708, MON87769)
R 602 25	**food**proof® GMO Soya Identification 2 LyoKit (BPS-CV127-9, DP-305423-1, DAS-68416-4, DAS-81419-2)
R 602 46	**food**proof® GMO Maize Identification 1 LyoKit (DAS-40278-9, LY038, VCO-01981-5)
Z 725 01	**food**proof® SL GMO Maize Multiplex Detection Kit (GA21, MIR604)
Z 725 02	**food**proof® SL GMO Maize Multiplex Detection Kit (MON89034, CBH351, Bt176)
Z 725 03	**food**proof® SL GMO Maize Multiplex Detection Kit (MON88017, NK603, MIR162)
Z 725 06	**food**proof® SL GMO Maize Multiplex Detection Kit (Bt11, TC1507)
Z 725 07	**food**proof® SL GMO Maize Multiplex Detection Kit (T25, MON810, MON863)

GMO Quantification Kits*

R 302 29	**food**proof® GMO 35S Maize Quantification Kit
R 302 16	**food**proof® GMO Bt176 Maize Quantification Kit
R 300 19	**food**proof® GMO RR Soya Quantification Kit - LC 1.x, 2.0, 480 -
R 302 19	**food**proof® GMO RR Soya Quantification Kit
R 302 35	**food**proof® GMO RR 2 Yield Soya Quantification Kit

** More Identification and Quantification Kits can be found on our website. Contact us for more information.*

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