



GMO analysis according to VLOG guidelines

The quality of food is becoming increasingly important to many consumers. To ensure the necessary transparency, i.a. the European Council Regulation (EC) (No. 834/2007) on organic production was implemented. This regulation regulates the use and labeling of genetically modified plants in food and feed (European labeling requirements 1829/2003 and 1830/2003).

Compliance with this basic organic regulation can be shown by a corresponding seal on the food products.

Other seals, such as the German organic seal or the seal of private associations can also be used.

The Association of Food without Genetic Engineering (VLOG e.V.), which emerged from the Federal Office of Consumer Protection and Food Safety (BMLV), offers the seal "Without genetic engineering" or "VLOG tested" if more stringent requirements are met (e.g. with the largely exclusion of genetically engineered feed).

This intends to enable consumers to have greater transparency and to ensure that genetically modified products have been virtually excluded across the entire food production process. To comply with this seal, defined measures, including defined analytical processes or methods, are specified.

Detailed information on VLOG certification and the required analytics can be found at: <u>https://ohnegentechnik.org/</u>

To meet these requirements, some of the SureFood[®] GMO kits have been adapted.

Sample amount: 2 grams

Usually 50 - 100 mg sample is used. A new additional kit (Art. No. S1055) enables the lysis of 2 grams sample material. The DNA preparation can be continued with the usual PREP Basic kit (Art. No. S1052).

Simplified in the summary, the identification follows differently depending on the matrix:

Soy and soy-containing feed	Kits recommended by R-Biopharm	Art. No.
Quantification of GTS 40-3-2 (Roundup Ready Soya)	GMO QUANT Roundup Ready Soya	S2014
Quantification of MON89788 (RR2 Soya)	GMO QUANT RR2Y Soya	S2029
Qualitative detection of A2704-12	GMO ID A2704-12 Soya	S2161
Optional: Detection using different screening kits and subsequent specific tests	GMO SCREEN 4plex 355/NOS/FMV+IAC GMO SCREEN 4plex BAR/NPTII/PAT/ CTP2:CP4 EPSPS	S2126 S2127
Corn and feed containing corn	Kits recommended by R-Biopharm	Art. No.
Screening auf 35S und NOS	GMO SCREEN 4plex 35S/NOS/FMV+IAC	S2126

Rapeseed and rapeseed feed	Kits recommended by R-Biopharm	Art. No.		
Screening NOS/PAT/EPSPS or FMV	GMO SCREEN 4plex 35S/NOS/FMV+IAC	S2126		
With a positive result each (NOS/PAT/CP2:CP4 EPSPS):				
MS8	GMO 4plex Canola II	S2167		
RF3	GMO 4plex Canola I	S2166		
T45	GMO 4plex Canola II	S2167		
GT73	GMO 4plex Canola II GMO QUANT GT73 Canola	S2167 S2061		

Identification of other potentially GMO-containing plant species	
GMO Plant 4plex Corn/Soya/Canola/Cotton	

identification of positive results:		
NK603	GMO 4plex Corn I	S2170
TC1507	GMO 4plex Corn I	S2170
		S2170 S2019
MON89034 + RR Soya	GMO 4plex Corn I	S2170

Rice and rice-containing foods	Kits recommended by R-Biopharm	Art. No.
 NOS, Cry1Ab/Ac	GMO SCREEN 4plex 35S/NOS/FMV+IAC GMO SCREEN 4plex BAR/PAT/CryIAb/ CTP2:CP4 EPSPS	S2126 S2128
 Construct-specific identification of laboratory/manufacturer	of positive results in consultation with the	
 e.g. GMO ID Bt63 Rice		S2024

S2156

Corresponding combinations apply to mixtures as described in detail in the VLOG guideline.

Salmon and salmon containing foods

A specific kit for salmon farmed in aquaculture tanks in the US is not available, but sequences have been published.

Debode F, Janssen E, Marien A, Devlin RH, Lieske K, Mankertz J, Berben G (2017) Detection of transgenic Atlantic and Coho salmon by real-time PCR. Food Analytical Methods, 11: (9), 2396-2406.

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Strategy recommended by R-Biopharm for analysis according to VLOG specifications

- DNA preparation from 2 gram sample using \$1055 and \$1052
 GMO screening kits with three master mixes/kits in parallel in the same run (or sequentially) with the same thermal profile (5 min = 95 ° C, 15 sec = 95 ° C, 30 sec = 60 ° C) and detection channels
- **B** From these results (no findings or various positive results), a targeted analysis for matrix/matrices and GMO events takes place

Detections channel/Kit	Art. No. S2126	Art. No. S2127	Art. No. S2128	Art. No. S2156
FAM	355	NPTII	CryIAb/IAc	Corn
HEX/VIC	IAC	PAT	PAT	Cotton
ROX	FMV	CTP2 CP4:EPSPS	CTP2:CP4 EPSPS	Rapeseed
Cy5	NOS	BAR	BAR	Soya

Suitable GMO kits for analysis of the minimum requirements according to the VLOG guideline

Name	Detection (sometimes different names common)	Event specific sequences/properties	Number of tests	Article number	For VLOG- analysis
DNA preparation					
PREP Basic	DNA preparation	For unprocessed food	100	S1052	VLOG
PREP Add-On	DNA preparation for 2 gram	Extension for 2 grams of food in combination with S1052	15	S1055	VLOG
Animal+Plant control 3plex	Extraction control	Plants/animal species/internal control	100	F4053	
GMO screening					
GMO SCREEN CaMV	CaMV control	Detection of the natural cauliflower mosaic virus (containing 355)	100	S2027	
GMO SCREEN 4plex 355/NOS/FMV+IAC	First screening	35S, NOS, FMV	100	52126	VLOG
GMO SCREEN 4plex BAR/NPTII/PAT/CTP2:CP4/ EPSPS	Second screening	BAR/NPTII/PAT/CTP2:CP4/EPSPS	100	52127	VLOG
GMO SCREEN 4plex BAR/PAT/CryIAb/ CTP2:CP4/EPSPS	Second screening	BAR/PAT/Cry1Ab/ CTP2:CP4/EPSPS	100	52128	VLOG
GMO Plant 4plex Corn/Soya/Canola/Cotton	Plant species screening	Corn, soybeans, canola, cotton	100	S2156	VLOG
Qualitative detection	·	·			
GMO ID 4plex Soya I	MON87708,CV127/DP305423/MON87701/ MON87769	Specific detection, events do not contain common screening elements	100	S2161	
GMO ID 4plex Soya II	RR-Soya , RR2Y-Soya, A2704-12 Soya, A5547-127 Soya	Specific detection, events do not contain common screening elements	100	S2162	
GMO ID 4plex Soya III	FG72, DAS68416, GMB151, DAS44406	Specific detection, events do not contain common screening elements	100	S2164	
GMO ID 4plex Soya IV	MON87705, DAS81419, MON87751, SYHT0H2	Specific detection, events do not contain common screening elements	100	S2165	
GMO ID 4plex EU Soya	DP305423, MON87769, CV127, MON87708, MON87701, DAS44406, DAS68416, FG72, GMB151, MON87705, MON87751, DAS81419, SYHT0H2, MON89788, A2704-12, GTS 40-3-2, A5547-35	Specific detection, events do not contain common scree- ning elements	100	52163	
GMO ID 4plex Corn I	MON810/MON89034/NK603/TC1507	Specific detection, event contains 35S, NOS, FMV, PAT, CTP2:CP4 EPSPS	100	52170	
GMO ID 4plex Canola I	MS8/GT73/T45	Specific detection, event contains NOS, BAR, 35S, PAT, FMV, CTP2:CP4 EPSPS	100	S2166	
GMO ID 4plex Canola II	MON88302/DP073496/RF3	Specific detection, event contains FMV, CTP2:CP4 EPSPS, NOS, BAR	100	S2167	
GMO ID Bt63 Rice	Bt63 Rice	Specific detection, event contains NOS	100	52024	
Quantitative detection					
GMO QUANT Roundup Ready Soya	GTS 40-4-2, RR1-S Roundup Ready Soya	Specific quantification, event contains 35S, NOS	2 x 50	52014	VLOG
GMO QUANT RR2Y Soya	Roundup Ready 2 Yield, RR2-S, RR2Y Soya	Specific quantification, event contains FMV, CTP2:CP4 EPSPS	2 x 50	52029	VLOG
GMO QUANT Bt176 Corn	Bt176 Corn	Specific quantification, event contains 35S, BAR	2 x 50	S2015	
GMO QUANT Bt11 Corn	Bt11 Corn	Specific quantification, event contains 35S, NOS, PAT	2 x 50	S2016	_
GMO QUANT T25 Corn	T25 Corn	Specific quantification, event contains 35S, PAT	2 x 50	S2017	
GMO QUANT MON810 Corn	MON810 Corn	Specific quantification, event contains 355	2 x 50	S2019	VLOG
GMO QUANT MON863 Corn	MON863 Mais	Specific quantification, event contains 35S, NOS, NPTII	2 x 50	S2051	
GMO QUANT MIR162 Corn	MIR 162 Corn	Specific quantification, event contains NOS	2 x 50	S2135	
GMO QUANT GT73 Canola	GT73 Canola	Specific quantification, event contains FMV, CTP2:CP4 EPSPS	2 x 50	S2061	VLOG