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Customer: 41119
Editor: Elke.Nuesseler
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Test report No. 17-001794 0020_V001 (this test report amends 17-001794 0020)

Sample receipt: 2017/05/24, Begin of analysis: 2017/05/24, End of analysis: 2017/05/26, Sample description: Genus: Petunia, Species: x atkinsiana, Variety Name: Amarena Twist, Breeder Code: 14PB224-05, Clone: 1, 2, Material: in vitro, For Customer: Kientzler, Sample code: 20

Sampling was carried out by the customer, who is responsible for representativeness, wrapping and logistic.
Wrapping: The sample was intact, there where no signs of manipulation.

GMO-Analysis qualitative (3-parameter: p35S, tNOS, pNOS-nptII) using Real Time-PCR					
Method	**Parameter**	**Result**	**LOD**	**LOQ**	
LP-P-01-41 2014-09	35S-Promoter-screening	**not detected**	0.01		%
LP-P-01-01 2001-05	NOS-Terminator-screening	**not detected**	0.01		%
LP-P-01-37 2009-09	pNOS-nptII-constructspec.	**not detected**	0.01		%
LP-P-01-03 2008-09	universal reference gene	**detected**	0.01		%

LOD = Limit of detection / Nachweisgrenze
LOQ = Limit of quantification / Quantifizierungsgrenze

The test is **positive** for: universal reference-gene.

At the specified LOD, the test is **negative** for: 35S-Promoter, NOS-Terminator, pNOS-nptII.

The detection of the universal reference gene demonstrates the presence of DNA in the sample which can be amplified.

Responsible for content: ppa Dr. K. Neumann

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Dr. K. Neumann: lab-manager; Dr. E. Nüßeler: assistant lab-manager; J. Kühnemann: Lab Specialist; M. Rampp: QSB

GMO-Analysis - qualitative Detection of genetically modified DNA

Using Real-Time PCR (polymerase chain reaction), DNA sequences frequently used in genetically modified plants can be detected. To prove the informational value of the PCR, an additional reference gene is analysed. This is a gene that is also found in NON-GMO plants. Analysis is done according to modifications of DIN 24276 in its current version.

Analysis method:

Homogenisation of solid samples: The amount named in the sample description is milled and homogenised.

DNA extraction method: The DNA is extracted using standard molecular biological methods (matrix dependent in-house methods; modifications of ISO 21571 in its current version). Depending on the matrix, between 400mg and 7500mg of the material is analysed (unless otherwise noted). For feed, seeds or inhomogeneous material at least 2500mg are used for the DNA extraction. For honey two times 50g are needed in order to enrich the pollen. Unless otherwise noted, all analyses are run **in duplicate.**

Qualitative detection using Real-Time PCR (45 cycles): Analysis is conducted using modifications of ASU L 00.00 122 (p35S + tNOS), protocols of the Joint Research Centre (JRC) of the European Commission, ASU L 15.06 1 (Bt63-rice), ASU L 23.04/03 1 (flax FP967), and the international norm ISO 21570 in its current version and according to publicly accessible publications.
Comment: The cauliflower mosaic virus is not a genetically modified organism. The 35S promoter is a part of this virus.

Controls and reference materials: For each analysis positive and negative controls were used. The negative control does not contain DNA and serves to demonstrate the lack of contaminants.

Limit of Detection (LOD): The detection limits were calculated for raw materials. Depending on the kind of sample matrix and degree of processing, the practical LOD can be higher.