JOINT RESEARCH CENTRE
Directorate F – Health and Food

REFERENCE MATERIAL CERTIFICATE

ERM®- BF421b

DRIED POTATO POWDER

Certified Values		
	Mass Fraction [2] [g/kg]	Uncertainty [2] [g/kg]
EH92-527-1 potato [1]	1000	0

1) Genetically modified potato with the unique identifier BPS-25271-9.

2) Certified values are values that fulfil the highest standards of accuracy. The value given is based on the experimental confirmation of the presence of the EH92-527-1 genetic modification in every individual potato used for the processing through colorimetric analysis of the amylose content in a cut tuber surface slice. The certified value and its uncertainty are traceable to the International System of Units (SI).

This certificate is valid for one year after purchase.

Sales date:

As the CRMs consist of genetically pure materials, no minimum sample intake is given. The sample intake is determined by the required specifications of the downstream use of the materials.

Accepted as an ERM®, Geel, September 2006
Latest Revision: June 2023

INFORMATION ONLY

Signed:

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DESCRIPTION OF THE MATERIAL

ERM-BF421a is one of two potato powder CRMs containing different mass fractions of genetically modified (GM) EH92-527-1 potatoes; ERM-BF421b is composed of milled, dried powder from purely EH92-527-1 potatoes and its genetic identity has been confirmed by nucleotide sequencing. ERM-BF421b is available in glass bottles containing approximately 0.5 g of potato powder closed under argon atmosphere. This reference material has been produced from whole tubers of genetically modified EH92-527-1 starch potato cultivar AMFLORA delivered by BASF Plant Science GmbH (Ludwigshafen, Germany). According to the information provided by BASF, one of the four genome copies in the tetraploid genetically modified EH92-527-1 potato carries the transgenic insert.

ANALYTICAL METHODS USED FOR CHARACTERISATION

The mass fraction is calculated based on the fact that each individual potato was tested for the presence of the genetic modification EH92-527-1 by staining a small potato piece for the presence of amylose (Lugol's test). The genetic identification is based on event-specific real-time PCR for EH92-527-1.

PARTICIPANTS

The following laboratories performed measurements in the scope of the homogeneity, stability and or characterisation study.
EC-DG JRC-IRMM, Geel (BE)

SAFETY INFORMATION

The usual laboratory safety precautions apply.

INTENDED USE

CRM ERM-BF421b shall only be used as reference material, providing the positive control for the detection of genetically modified EH92-527-1 potato in food and feed.

INSTRUCTIONS FOR USE

The customer is strongly advised to read the certification report with regard to the extraction of genomic DNA from this CRM. A short guideline for the purification of genomic DNA from this CRM is here included as an Annex.
Dispose in accordance with good laboratory practice.
Please note that repeated sampling or use has not been tested and occurs under the responsibility of the user.
For general information on handling of reference materials, please see ERM Application Note 6, available on https://crm.jrc.ec.europa.eu/e/132/User-support-Application-Notes .

STORAGE

Bottles should be stored dry and in the dark at maximum 4 °C. The powder is hygroscopic and should be used immediately after opening.
Please note that the stability of samples after opening has not been tested. The European Commission cannot be held responsible for changes that happen to samples after opening or when the material is stored differently from the stated storage conditions at the customer's premises.
For more information regarding the shelf life of reference materials please see ERM Application Note 7, available on https://crm.jrc.ec.europa.eu/e/132/User-support-Application-Notes .

LEGAL NOTICE

NOTE

A detailed certification report is available at https://crm.jrc.ec.europa.eu/.

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Annex: Guidelines for the extraction of genomic DNA from ERM-BF421

ERM-BF421 forms a set of CRMs consisting of dried powder made from starch potatoes. The high starch content in the powder affects the DNA extractability and the quality of the genomic DNA obtained. When aqueous suspensions of this powder are heated above 55 °C, the starch will swell, resulting in a viscous or gelatinous solution; this swelling effect is more pronounced in ERM-BF421b than in ERM-BF421a powder. The mass fraction of DNA extracted from ERM-BF421b may therefore be lower than that from ERM-BF421a.

The following procedure is given as a guideline for DNA extraction from these CRMs. Results obtained with this modified CTAB method can be found in the certification report.

Because of the observed differences in the mass fraction of extractable genomic DNA between ERM-BF421a and ERM-BF421b, the customer is strongly advised not to prepare gravimetric mixtures of these CRMs and to use the purified DNA from such mixtures for calibration purposes.

Table: DNA extraction protocol for ERM-BF421 potato powder

Step	Details
Weighing	200 mg (ERM-BF421a) or 150 mg (ERM-BF421b) powder
Lysis	Add 1 mL CTAB extraction buffer[1] at 65 °C, 10 µL RNase A (100 mg/mL, Qiagen # 19101) and 20 µL Proteinase K (20 mg/mL, Qiagen # 19133), mix by pipetting up and down and shaking[2].
	Incubate 30 min at 65 °C with continuous agitation and regular inversion of the tubes, ensuring adequate mixing of the suspension.
	Spin down cell debris by centrifugation (10 min, 13000 rpm) and decant supernatant into a new tube.
Chloroform extraction (2X)	Add an equal volume of chloroform, mix, centrifuge to separate phases, transfer upper phase to a new tube; repeat once.
DNA precipitation	Add 2.2 volumes CTAB precipitation buffer[3], incubate 1 h at room temperature, spin down DNA pellet (10 min, 13000 rpm) and discard supernatant.
Resuspension + chloroform extraction	Resuspend DNA pellet in 400 µL 1.2 mol/L NaCl, extract once with equal volume of chloroform, centrifuge to separate phases, transfer upper phase to new tube.
Ethanol precipitation	Add 2 volumes cold (-20 °C) ethanol, mix well, and incubate at - 20 °C if no precipitate seen (at least 10 min); pellet DNA by centrifugation, wash with cold 70 % (v/v) ethanol and air-dry the pellet.
Resuspension	Resuspend the DNA in nuclease-free water, preferably by overnight incubation at 4 °C, then agitate 30 min at 37 °C before use[4]

1 CTAB extraction buffer: 1.4 % (m/v) CTAB (cetyltrimethylammonium bromide), 1 mol/L NaCl, 0.1 mol/L Tris-HCl, 0.15 mol/L Na_2EDTA, pH 8.0

2 Although not necessary, the addition of 1 µL of a heat-stable α-amylase (e.g. Sigma # A3403, 786 units/mg) reduces starch swelling and results in an increased DNA recovery.

3 CTAB precipitation buffer: 0.5 % (m/v) CTAB, 0.04 mol/L NaCl, 0.05 mol/L Tris-HCl pH 8.0

4 Further purification of the DNA extract, e.g. on a silica or ion-exchange column, may be necessary for certain applications.