PRODUCT INFORMATION SHEET

ALM Verification: Gluten in "gluten-free" Cocoa Biscuit Matrix

5 Samples containing Wheat Flour (levels: 2,0 / 10 / 20 / 50 / 100 mg/kg)

PT originated REFERENCE MATERIAL (RM)

Description of the RM

Name / Material of related PT	*DLA ALM2 (2021) ALM-Verification: Gluten, 5 Samples containing Gluten in „gluten-free" Cocoa Biscuit Matrix (and a „blank sample")*
Identifier / LOT	*RM 2021-ALM2 Set*
Expiry date	*Mai 2027*
Matrix	*Cocoa Biscuit Matrix*
Physical description	*Powder*
Packaging / weight	*Sealed foil pouche / 20 g*
Storage conditions	*2 - 10°C, long term < -18°C (dry and dark)*
Name and address manufacturer	***DLA - Proficiency Tests GmbH*** *Hauptstr. 80, 23845 Oering, Germany* *Tel. ++49-(0)4532-9183358, Fax. ++49(0)4102-9944976* *eMail. pt@dla-lvu.de, website. www.dla-lvu.de*

Composition of Material

PT-Sample series	**Level 0** **„blank"**	**Level 1** **2 mg/kg**	**Level 2** **10 mg/kg**	**Level 3** **20 mg/kg**	**Level 4** **50 mg/kg**	**Level 5** **100 mg/kg**
Ingredients	**g/100 g**	**g/100g**	**g/100g**	**g/100g**	**g/100g**	**g/100g**
Cocoa Biscuits, gluten-free (baked 150°C, 30 min) Ingredients: Teff flour (dwarf millet), sugar, margarine (sunflower oil, cocnut fat and additives), cocoa powder (4,6%), rice protein, salt	100	99,6	98,0	96,0	90,0	80,0
Cocoa Biscuits, spiked (baked 150°C, 30 min) Ingredients: Teff flour (dwarf millet), sugar, margarine (sunflower oil, coconut fat and additives), cocoa powder (4.6%), rice protein, salt, wheat-flour-mixture(25% in dry matter)	-	0,40	2,0	4,0	10,0	20,0
Allergen-Contents	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
thereof Wheat: *Wheat flour mixture (21 products from Europe, Asia, USA)* – Wheat flour* – *with 10% protein***	-	23,2 2,34	115 11,6	229 23,2	573 57,9	1145 116
*- thereof **Gluten****	**-**	**2,02**	**9,98**	**20,0**	**49,9**	**99,6**
Extended combined uncertainty (k=2) of Gluten content (= ± 12 %)		± 0,24	± 1,2	± 2,4	± 6,0	± 12

*Allergen contents as „total food" as described in column ingredients according to gravimetric mixture
** Protein contents according to laboratory analysis of raw material: 10,1 ± 0,17% (total nitrogen according to Kjeldahl with F=5,7 for wheat protein)
*** Protein contents according to literature values (approx. 8,7% gluten in wheat flour)

Minimum Sample Size

A minimum sample size of 5 g is recommended for sample preparation.
The homogeneity tests were performed with sample sizes of 5 g (Microtracer analysis).
The mixture homogeneity before bottling was examined 8-fold by microtracer analysis. Homogeneity was sufficient with a probability of ≥ 25% (excellent mixture) and a HorRat value in the range of acceptance (0,3 to 1,3) (for more details see final report of PT).

Reference Values

All reference values were derived from the related proficiency test (PT) round (for more details see final report).

Reference Values qualitative ELISA

Evaluation number	Level 0 „Null“	Level 1 2 mg/kg	Level 2 10 mg/kg	Level 3 (Action Level) 20 mg/kg	Level 4 50 mg/kg	Level 5 100 mg/kg
	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg
Number positive	0	6	10	10	10	10
Number negative	10	4	0	0	0	0
Percent positive	0	60	100	100	100	100
Percent negative	100	40	0	0	0	0
Consensus value	negative	none	positive	positive	positive	positive
Spiking	negative	positive	positive	positive	positive	positive

Methods:
AQ-G12 = AgraQuant, RomerLabs
IL = Immunolab
RS = Ridascreen®, R-Biopharm
SP-R5 = SensiSpec INgezim Gluten R5, Eurofins
VT-R5 = Veratox, Neogen

Reference Values qualitative PCR

Evaluation number	Level 0 „Null“	Level 1 2 mg/kg	Level 2 10 mg/kg	Level 3 (Action Level) 20 mg/kg	Level 4 50 mg/kg	Level 5 100 mg/kg
	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg
Number positive	0	2	2	2	2	2
Number negative	2	0	0	0	0	0
Percent positive	0	100	100	100	100	100
Percent negative	100	0	0	0	0	0
Consensus value	negative	positive	positive	positive	positive	positive
Spiking	negative	positive	positive	positive	positive	positive

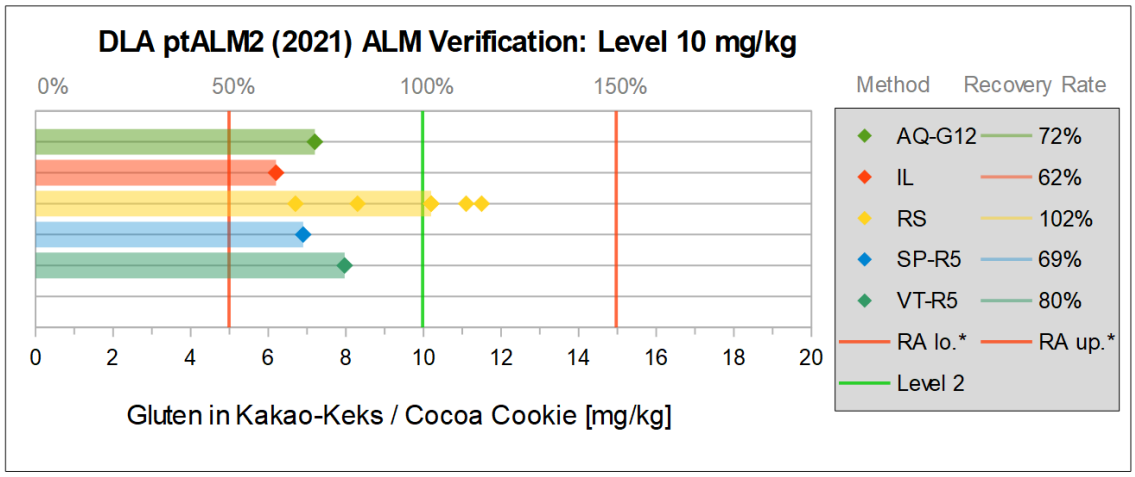
Methods:
SFA = Sure Food ALLERGEN, R-Biopharm / Congen

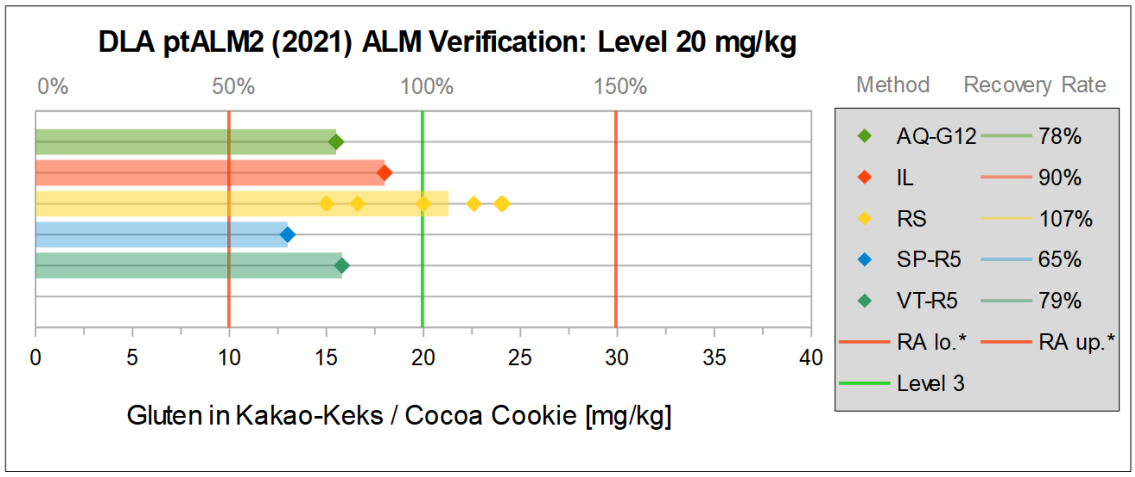
Reference Values quantitative in mg/kg ELISA

Parameter	Gluten	
	Level 2 (10,0 mg/kg)	
	all Methods	Method RS
Numbers of results	10	6
Number of outliers	0	0
Assigned Value (Xpt)	***8,63***	***9,67***
Robust standard deviation	2,22	2,07
Standard uncertainty	0,877	1,06
Target standard deviation σpt	2,16	2,42
Target range	***4,31 – 12,9***	***4,83 – 14,5***

Methods *RS = R-Biopharm, Ridascreen®*

Median of recovery rates: levels 2, 3 and 4





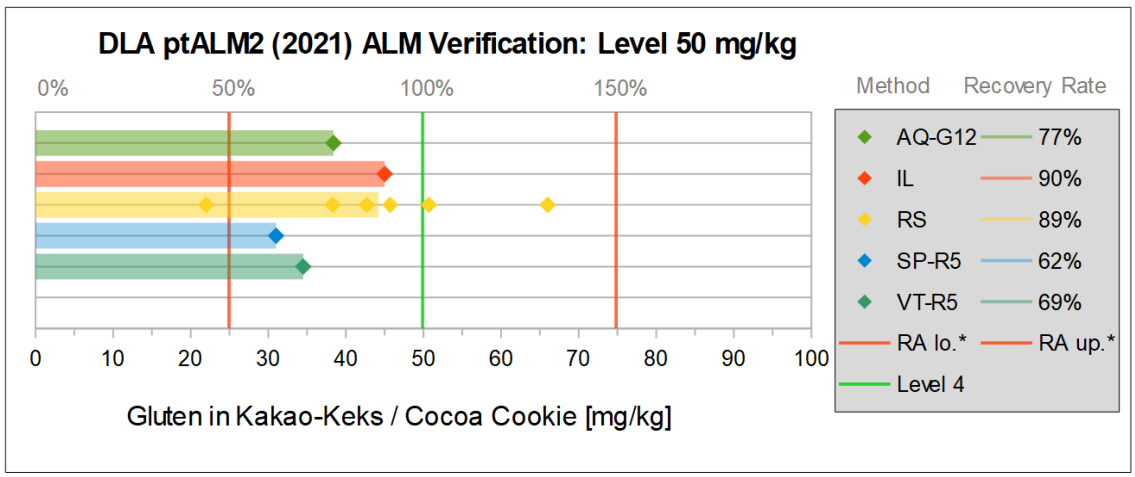


Abb./Fig. 2: Graphs of single results (Level 2-4) separated by methods with corresponding mean recovery rates, lower scale gluten content in mg/kg, upper scale recovery rate in % with * range of acceptance from 50% - 150% (* range of acceptance: RA lower limit to RA upper limit)

Intended Use

- for laboratory use only, no food!
- validation of analytical methods
- control sample for quality control charts
- test material for inter-laboratory comparisons

Storage Instructions and Handling Information

The material should be stored as stated on page 1. Before use of the material should be warmed up to room temperature. The content should be mixed thoroughly prior to subsampling (see minimum sample size above). The material should be weighed immediately after opening of the foil pouche/bottle and closed again afterwards in order to avoid uptake of moisture. Frequent freezing and thawing should be avoided as this can lead to changes in the properties of the material. The expiry date of this RM is based on the current knowledge and applies only for proper storage conditions in the originally closed foil pouche/bottle.

Safety Precautions

The usual laboratory safety precautions should be applied when working with the RM considering the type of ingredients, parameters and its concentration.

Data Source

The material was analyzed by 10 laboratories within the related proficiency test (PT). Different analytical methods have been used.

Various

The user must determine the suitability of this RM for its particular purpose. DLA makes no warranty of any kind, expressed or implied, other than its products meet all quality control standards set by DLA. We do not guarantee this product can be used for a special application.

DLA Proficiency Tests

approved (PT-Coordinator)	updated
Matthias Besler-Scharf, PhD	Ulrike Vorreiter
signed M. Besler-Scharf	*signed U. Vorreiter*
26 July 2023	27 May 2026