

## PRODUCT INFORMATION SHEET

**Peanut, Walnut and Milk**  
**in Cocoa Biscuit (baked) (positive)**  
PT originated REFERENCE MATERIAL (RM)

**Description of the RM**

|                               |                                                                                                                                                                                    |
|-------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Name / Material of related PT | DLA ptAL05 (2024) – Allergens V: Peanut, Walnut and Milk in Cocoa Biscuit (baked) with "Spiking Level Sample" (3 different Samples) – <b>Sample A</b> (positive sample)            |
| Identifier / LOT              | RM 2024-AL05 A                                                                                                                                                                     |
| Expiry date                   | December 2028                                                                                                                                                                      |
| Matrix                        | Cocoa Biscuit (baked)                                                                                                                                                              |
| Physical description          | Powder                                                                                                                                                                             |
| Packaging / weight            | Sealed foil poche / 25 g                                                                                                                                                           |
| Storage conditions            | 2 - 10°C (dry and dark)                                                                                                                                                            |
| Name and address manufacturer | <b>DLA - Proficiency Tests GmbH</b><br>Hauptstr. 80, 23845 Oering, Germany<br>Tel. ++49-(0)4532-9183358, Fax. ++49(0)4102-9944976<br>eMail. pt@dla-lvu.de, website. www.dla-lvu.de |

**Composition of Material**

| Ingredients                                                                                                                                                                                                                                                                   | Sample A<br>present sample                           | Sample B    | Sample SP                                            |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|-------------|------------------------------------------------------|
| <b>Cocoa biscuits, gluten-free (baked 150°, 30 min)</b><br>Ingredients: Teff flour (dwarf millet), sugar, margarine (sunflower oil, coconut fat and additives), cocoa powder (4.6%), rice protein, salt                                                                       | 96,0 g/100 g                                         | 100 g/100 g | -                                                    |
| <b>Cocoa biscuit, gluten-free, spiked (baked 150°, 30 min)</b><br>Ingredients: Teff flour (dwarf millet), sugar, margarine (sunflower oil, coconut fat and additives), cocoa powder (4.6%), rice protein, salt and<br><b>positive sample:</b> allergenic ingredients as below | 4,03 g/100 g                                         | -           | -                                                    |
| <b>Potato Powder</b> Ingredients: Potatoes, E471, E304, E223, E100                                                                                                                                                                                                            | -                                                    | -           | 99,9 g/100 g                                         |
| <b>Peanut, roasted, milled</b> , mixture (18 products from USA, Asia, Africa, South America)<br>– as peanut*<br>– thereof 23,2% total protein**                                                                                                                               | 20,3 mg/kg<br>4,70 mg/kg                             | -           | 21,2 mg/kg<br>4,91 mg/kg                             |
| <b>Walnut, roasted, milled</b> , mixture (11 products i.a. from North America, South America, Europe)<br>– as walnut*<br>– thereof 15,8% total protein**                                                                                                                      | 20,6 mg/kg<br>3,24 mg/kg                             | -           | 21,0 mg/kg<br>3,30 mg/kg                             |
| <b>Skimmed milk powder</b> , mixture (9 products from Europe, USA)<br>– as skimmed milk powder*<br>– thereof 33,0% total protein**<br>– thereof casein***<br>– thereof $\beta$ -lactoglobulin***                                                                              | 20,1 mg/kg<br>6,61 mg/kg<br>5,29 mg/kg<br>0,66 mg/kg | -           | 25,0 mg/kg<br>8,25 mg/kg<br>6,60 mg/kg<br>0,83 mg/kg |
| <b>further Ingredients:</b> Maltodextrin, sodium sulfate and silicon dioxide                                                                                                                                                                                                  | <0,2 g/100 g                                         | -           | <0,2 g/100 g                                         |

\* Allergen contents as "total food" as described in the column ingredients according to the gravimetric mixture

\*\* Protein contents according to laboratory analysis of the raw material (total nitrogen according to Kjeldahl with F=5,46 for peanut protein, F=5,30 for walnut protein and F=6,38 for milk protein)

\*\*\* Protein contents according to literature values (approx. 80% casein and 10%  $\beta$ -lactoglobulin in total milk protein)

**Note:** The metrological traceability of temperature, mass and volume during production of the PT samples is ensured by DAkkS-compliant calibrated reference materials.

### Minimum Sample Size / Homogeneity

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) and 1 - 5 g (ELISA methods), respectively.

The mixture homogeneity was examined 10-fold by microtracer analysis. Homogeneity was sufficient with a probability of  $\geq 25\%$  (excellent mixture) and a HorRat value in the range of acceptance (0,3 to 1,3). The homogeneity tests of the parameters were carried out by ELISA analyzing two subsets from 10 test items (according to ISO 13528:2015 Annex B). Homogeneity was sufficient with a standard deviation between the samples  $S_s$  of  $\leq 15\%$  („heterogeneity standard deviation“) using ELISA test kits from different manufacturers, e.g. AgraQuant Allergen ELISA Romer Labs, GSD Allergen ELISA Gold Standard Diagnostics Kassel, Morinaga Food Allergen ELISA Kits MloBS, Veratox ELISA for Food Allergens Neogen (for more details see final report of PT).

### Reference Values

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

### Reference Values quantitative in mg/kg ELISA

| Parameter                               | Peanut             |                    |
|-----------------------------------------|--------------------|--------------------|
|                                         | All Results*       | Method RS**        |
| Number of results                       | 31                 | 17                 |
| Number of outliers                      | 0                  | 0                  |
| <b>Assigned Value (X<sub>pt</sub>)</b>  | <b>14,7</b>        | <b>15,7</b>        |
| Robust standard deviation               | 4,13               | 4,96               |
| Standard uncertainty                    | 0,927              | 1,50               |
| Target standard deviation $\sigma_{pt}$ | 3,67               | 3,93               |
| <b>Target range</b>                     | <b>7,35 – 22,0</b> | <b>7,87 – 23,6</b> |

\* ELISA Method: divers Methods

\*\* ELISA Method: RS = Ridascreen®, R-Biopharm

| Parameter                               | Walnut             |                    |                    |
|-----------------------------------------|--------------------|--------------------|--------------------|
|                                         | Method "Peak 3"*   | Method "Peak 14"** | Method AQ***       |
| Number of results                       | 13                 | 6                  | 5                  |
| Number of outliers                      | 0                  | 0                  | 0                  |
| <b>Assigned Value (X<sub>pt</sub>)</b>  | <b>3,19</b>        | <b>13,7</b>        | <b>2,99</b>        |
| Robust standard deviation               | 0,860              | 1,50               | 0,672              |
| Standard uncertainty                    | 0,298              | 0,765              | 0,376              |
| Target standard deviation $\sigma_{pt}$ | 0,799              | 3,43               | 0,747              |
| <b>Target range</b>                     | <b>1,60 – 4,79</b> | <b>6,87 – 20,6</b> | <b>1,49 – 4,48</b> |

\* ELISA Method: Methods "Peak 3": AQ = AgraQuant, RomerLabs, BC = BioCheck ELISA, BI = Allergen ELISA, BioSystems S.A., BK = BioKits, Neogen, GSD = ELISA, Gold Standard Diagnostics, Kassel, NL = nutriLinia® Allergen-ELISA, SP = SensiSpec ELISA Kit, Gold Standard Diagnostics

\*\* ELISA Method: Methods "Peak 14": 3M = 3M Protein ELISA Kit, BF = MonoTrace ELISA, BioFront Technologies, NL-E = nutriLinia®E Allergen-ELISA

\*\*\* ELISA Method: AQ = AgraQuant, RomerLabs

| Parameter                               | Milk Protein        |                     |                     |
|-----------------------------------------|---------------------|---------------------|---------------------|
|                                         | All Results*        | Method AQ-M**       | Method RS-FM***     |
| Number of results                       | 23                  | 4                   | 6                   |
| Number of outliers                      | 0                   | 0                   | 0                   |
| <b>Assigned Value (X<sub>pt</sub>)</b>  | <b>1,11</b>         | <b>0,765</b>        | <b>1,75</b>         |
| Robust standard deviation               | 0,666               | 0,0814              | 1,26                |
| Standard uncertainty                    | 0,174               | 0,0509              | 0,645               |
| Target standard deviation $\sigma_{pt}$ | 0,278               | 0,191               | 0,438               |
| <b>Target range</b>                     | <b>0,555 – 1,67</b> | <b>0,382 – 1,15</b> | <b>0,875 – 2,63</b> |

\* ELISA Method: divers Methods

\*\* ELISA Method: Methods AQ-M = AgraQuant Milk Protein, RomerLabs

\*\*\* ELISA Method: RS-FM = Ridascreen® Fast Milk, R-Biopharm

## Reference Values qualitative ELISA

| Parameter              | Peanut*         | Walnut*         | Milk Protein* |
|------------------------|-----------------|-----------------|---------------|
| Number positive        | 34              | 20              | 26            |
| Number negative        | 0               | 0               | 18            |
| Percent positive       | 100             | 100             | 59            |
| Percent negative       | 0               | 0               | 41            |
| <b>Consensus value</b> | <b>positive</b> | <b>positive</b> | <b>none**</b> |

\* Results of divers ELISA Methods (LODs positive results Peanut 0,1-1 mg/kg, Walnut 0,2-2 mg/kg, Milk Protein 0,05-2,5 mg/kg)

\*\* No consensus value of  $\geq 75\%$  positive results was obtained.

## Reference Values quantitative in mg/kg PCR

| Parameter                               | Peanut*             |
|-----------------------------------------|---------------------|
| Number of results                       | 6                   |
| Number of outliers                      | 0                   |
| <b>Assigned Value (X<sub>pt</sub>)</b>  | <b>1,75</b>         |
| Robust standard deviation               | 0,920               |
| Standard uncertainty                    | 0,470               |
| Target standard deviation $\sigma_{pt}$ | 0,438               |
| <b>Target range</b>                     | <b>0,875 – 2,63</b> |

\* PCR Method: divers Methods

## Reference Values qualitative PCR

| Parameter              | Peanut*         | Walnut*       |
|------------------------|-----------------|---------------|
| Number positive        | 16              | 13            |
| Number negative        | 4               | 6             |
| Percent positive       | 80              | 68            |
| Percent negative       | 20              | 32            |
| <b>Consensus value</b> | <b>positive</b> | <b>none**</b> |

\* Results of diverse PCR Methods (LODs positive results Peanut 0,4-2 mg/kg, Walnut 0,4 mg/kg)

\*\* No consensus value of  $\geq 75\%$  positive results was obtained.

#### **Intended Use**

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

#### **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 45 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.



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