

## PRODUCT INFORMATION SHEET

## Cashew, Milk and Peanut in Cocoa Biscuit (positive)

PT originated REFERENCE MATERIAL (RM)

**Description of the RM**

|                               |                                                                                                                                                                                    |
|-------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Name / Material of related PT | DLA ptAL05 (2025) – Allergens VII: Cashew, Milk and Peanut in Cocoa Biscuit with "Spiking Level Sample" (3 different Samples) – <b>Sample B</b> (positive sample)                  |
| Identifier / LOT              | RM 2025-AL05 B                                                                                                                                                                     |
| Expiry date                   | December 2026                                                                                                                                                                      |
| Matrix                        | Cocoa Biscuit                                                                                                                                                                      |
| Physical description          | Powder                                                                                                                                                                             |
| Packaging / weight            | Sealed foil poche / 25 g                                                                                                                                                           |
| Storage conditions            | < -18°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    | Sample B<br>present sample                           | Sample SP                                            |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------|------------------------------------------------------|------------------------------------------------------|
| <b>Cocoa biscuits, gluten-free (baked 150°, 30 min)</b><br>Ingredients: Teff flour (dwarf millet), sugar, margarine (vegetable oils and additives), cocoa powder (5%), rice protein, salt                                                                     | 100 g/100 g | 94,2 g/100 g                                         | -                                                    |
| <b>Cocoa biscuit, gluten-free, spiked (baked 150°, 30 min)</b><br>Ingredients: Teff flour (dwarf millet), sugar, margarine (vegetable oils and additives), cocoa powder (5%), rice protein, salt and <b>positive sample</b> : allergenic ingredients as below | -           | 5,81 g/100 g                                         | -                                                    |
| <b>Potato Powder</b><br>Ingredients: Potatoes, E471, E304, E223, E100                                                                                                                                                                                         | -           | -                                                    | 99,8 g/100 g                                         |
| <b>Cashew, roasted, milled</b> , mixture (9 products from Asia and unknown origin)<br>– as cashew*<br>– thereof 18,5% total protein**                                                                                                                         | -           | 30,6 mg/kg<br>5,66 mg/kg                             | 34,6 mg/kg<br>6,40 mg/kg                             |
| <b>Milk: 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***                                                        | -           | 30,3 mg/kg<br>10,0 mg/kg<br>8,00 mg/kg<br>1,00 mg/kg | 33,6 mg/kg<br>11,1 mg/kg<br>8,86 mg/kg<br>1,11 mg/kg |
| <b>Peanut, roasted: milled</b> , mixture (18 products from USA, Asia, Africa, South America)<br>– as peanut*<br>– thereof 23,2% total protein**                                                                                                               | -           | 31,1 mg/kg<br>7,22 mg/kg                             | 32,5 mg/kg<br>7,54 mg/kg                             |
| <b>further Ingredients:</b><br>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=5,3 for cashew protein, F=6,38 for milk protein and F=5,46 for peanut 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 MIOBS, Veratox ELISA for Food Allergens Neogen (for more details see final report of PT).

### Reference Values

All reference values were derived from the related ISO 17043 accredited PT round (for more details see final report).

### Reference Values quantitative in mg/kg ELISA

| Parameter                               | Cashew             |                    |                    |                    |
|-----------------------------------------|--------------------|--------------------|--------------------|--------------------|
|                                         | All Results*       | Method BF**        | Method RS-F***     | Method SP****      |
| Number of results                       | 26                 | 5                  | 7                  | 5                  |
| Number of outliers                      | -                  | -                  | -                  | -                  |
| <b>Assigned Value (X<sub>pt</sub>)</b>  | <b>13,6</b>        | <b>12,0</b>        | <b>14,9</b>        | <b>14,5</b>        |
| Robust standard deviation               | 4,27               | 3,69               | 6,05               | 2,64               |
| Standard uncertainty                    | 1,05               | 2,06               | 2,86               | 1,47               |
| Target standard deviation $\sigma_{pt}$ | 3,39               | 3,00               | 3,72               | 3,62               |
| <b>Target range</b>                     | <b>6,78 – 20,3</b> | <b>6,00 – 18,0</b> | <b>7,44 – 22,3</b> | <b>7,24 – 21,7</b> |

\* ELISA Method: divers Methods

\*\* ELISA Method: BF = MonoTrace ELISA, BioFront Technologies

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

\*\*\*\* ELISA Method: SP = SensiSpec ELISA Kit, Gold Standard Diagnostics

| Parameter                     | Milk (as Milk Protein)* |                     |                    |
|-------------------------------|-------------------------|---------------------|--------------------|
|                               | All Results (Info)*     | Method Peak 1,5**   | Method RS-FM***    |
| Number of results             | 27                      | 15                  | 12                 |
| Number of outliers            | -                       | -                   | -                  |
| <b>Assigned Value (Xpt)</b>   | <b>2,42</b>             | <b>1,53</b>         | <b>3,40</b>        |
| Robust standard deviation     | 1,34                    | 0,582               | 0,907              |
| Standard uncertainty          | 0,322                   | 0,188               | 0,327              |
| Target standard deviation opt | 0,604                   | 0,838               | 0,850              |
| <b>Target range</b>           | <b>1,21 – 3,63</b>      | <b>0,765 – 2,30</b> | <b>1,70 – 5,10</b> |

\* ELISA Method: divers Methods

\*\* ELISA Method: "Peak 1,5" methods = AQ-M = AgraQuant, RomerLabs, ES-M = ELISA-Systems, GSD-M = ELISA, Gold Standard Diagnostics, Kassel, MI-IIM = Morinaga ELISA Kit II, SP-M = SensiSpec ELISA Kit, Gold Standard Diagnostics and VT = Veratox, Neogen

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

(Info): for information only

| Parameter                     | Milk (as Casein)    | Milk (as $\beta$ -Lactoglobulin) | Peanut             |                    |
|-------------------------------|---------------------|----------------------------------|--------------------|--------------------|
|                               | All Results (Info)* | All Results (Info)*              | All Results*       | Method RS**        |
| Number of results             | 17                  | 6                                | 29                 | 15                 |
| Number of outliers            | -                   | -                                | -                  | -                  |
| <b>Assigned Value (Xpt)</b>   | <b>1,78</b>         | <b>0,232 ***</b>                 | <b>39,1</b>        | <b>39,5</b>        |
| Robust standard deviation     | 1,09                | 0,211                            | 8,04               | 4,48               |
| Standard uncertainty          | 0,332               | -                                | 1,87               | 1,45               |
| Target standard deviation opt | 0,444               | -                                | 9,78               | 9,88               |
| <b>Target range</b>           | <b>0,889 – 2,67</b> | <b>-***</b>                      | <b>19,6 – 58,7</b> | <b>19,8 – 59,3</b> |

\* ELISA Method: divers Methods

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

\*\*\* no statistical evaluation, robust mean for information only

(Info): for information only

### Reference Values qualitative ELISA

| Parameter              | Cashew*         | Milk (as Milk Protein)* | Milk (as Casein)* | Milk (as $\beta$ -Lactoglobulin)* | Peanut          |
|------------------------|-----------------|-------------------------|-------------------|-----------------------------------|-----------------|
| Number positive        | 26              | 28                      | 18                | 6                                 | 32              |
| Number negative        | 0               | 6                       | 0                 | 2                                 | 0               |
| Percent positive       | 100             | 82                      | 100               | 75                                | 100             |
| Percent negative       | 0               | 18                      | 0                 | 25                                | 0               |
| <b>Consensus value</b> | <b>positive</b> | <b>positive</b>         | <b>positive</b>   | <b>positive</b>                   | <b>positive</b> |

\* Results of diverse ELISA Methods (LODs positive results Cashew 0,12-2 mg/kg, Milk Protein 0,05-2,5 mg/kg, Casein 0,06-2,5 mg/kg,  $\beta$ -Lactoglobulin 0,003-1,4 mg/kg, Peanut 0,1-1)

### Reference Values quantitative PCR

| Parameter                     | Peanut              |
|-------------------------------|---------------------|
|                               | All Results (Info)* |
| Number of results             | 6                   |
| Number of outliers            | -                   |
| <b>Assigned Value (Xpt)</b>   | <b>5,91</b>         |
| Robust standard deviation     | 2,81                |
| Standard uncertainty          | 1,43                |
| Target standard deviation opt | 1,39                |
| <b>Target range</b>           | <b>2,78 – 8,34</b>  |

\* Results of diverse PCR Methods

(Info): for information only

## Reference Values qualitative PCR

| Parameter              | Cashew*         | Peanut*         |
|------------------------|-----------------|-----------------|
| Number positive        | 9               | 15              |
| Number negative        | 1               | 2               |
| Percent positive       | 90              | 88              |
| Percent negative       | 10              | 12              |
| <b>Consensus value</b> | <b>positive</b> | <b>positive</b> |

\* Results of diverse PCR Methods (LODs positive results Cashew 1,0-10 mg/kg, Peanut 0,4 -10mg/kg)

## 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 poche/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 poche/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.



| created                    | approved (PT-Coordinator)      | updated |
|----------------------------|--------------------------------|---------|
| Ulrike Vorreiter           | Matthias Besler-Scharf, PhD    | -       |
| <i>signed U. Vorreiter</i> | <i>signed M. Besler-Scharf</i> | -       |
| 21 January 2026            | 6 February 2026                | -       |