Proteon

Milk Express

Technical Report

ISO 9001
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Certification

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1. PRINCIPLE OF THE TEST

PROTEON MILKEXPRESS is an immunochromatographic assay for the detection of milk in food samples and work surfaces. β-Lactoglobulin (β-LG) along with caseins, a-lactalbumin and serum albumin are the main allergens in milk. Therefore, the identification of these substances is very important to avoid allergic reactions.

The assay is based on the detection of β-LG, which reacts on the test strip with a specific antibody bound to red particles. In this way, the appearance of a line of this color on the test strip corresponds to a positive result. The blue line indicates the correct development of the test.

2. TEST PROCEDURE

Detailed information on the procedure is available in the product script.

- PROTEON MILK EXPRESS (25 tests): G-COM-PR.15 - Revision 14
- PROTEON MILK EXPRESS (10 test): G-COM-PR.46 - Revision 2
- PROTEON MILK EXPRESS SW – G-COM-PR.31 - Revision 5

3. TECHNICAL SPECIFICATIONS

The validation tests were carried out according to the AOAC 2016 guidelines. ZEULAB, as a manufacturer, validates its tests according to international reference guides (1). All its batches undergo rigorous controls to guarantee product specifications. The user must and can verify that the tests work properly in their work environment by following ZEULAB's technical recommendations.

Immunochromatographic tests are qualitative tests with a very simple test protocol. It is advisable to carry out an initial validation that considers aspects such as matrix, specificity and sensitivity (ask the manufacturer for advice). Periodically, the user must verify that the system is working correctly. ZEULAB can supply and/or recommend how to prepare these controls according to the test specifications.

3.1. Limit of Detection (LOD)

To calculate the LOD of β-lactoglobulin, the Probability of Detection (POD) statistical method was used. As described by the guidelines, 20 replicates were made for each concentration using one batch until the probability of detection was 1.0. For concentrations greater than the LOD, only 6 replicates are necessary. To carry out these tests, the instructions described in the kit manual were followed. The results are shown in Table 1.

Table 1. Probability of Detection (POD)

β-LG (ppm)	Milk protein (ppm)	Number of repetitions	P.O.D.
0	0	20	0.0
0.03	0.3	20	0.4
0.06	0.6	20	0.5
0.3	3.0	20	1.0
0.6	6.0	20	1.0
1	10	6	1.0
5	50	6	1.0
10	100	6	1.0

As can be seen in Table 1, the LOD of β-LG is 0.3 ppm (POD=1.0, 20 replicates). The 20 repetitions carried out with the batch for the 0.6 ppm concentration confirmed this level of detection.
To obtain the concentration of skimmed milk powder (ppm) detected by the kit, the concentration of β-LG was multiplied by a conversion factor of 10, being 3 ppm (2).

3.2. Specificity

To determine the specificity of the test, different protein extracts and ingredients were analyzed (see Table 2). Samples containing β-LG concentrations below the detection limit were identified as negative. In these samples, only the control line (blue) appeared in the central part of the strip. All samples tested, except for casein and whey powder, gave negative results. This kit is capable of identifying samples that contain the β-LG protein, and gives a negative result with other proteins and ingredients that should not contain it.

Table 2. Specificity

Ingredient	Result	Ingredient	Result	Ingredient	Result
Raw almond	NEGATIVE	Walnut	NEGATIVE	Sesame	NEGATIVE
Toasted almond	NEGATIVE	Raw Fish	INVALID	Buckwheat	NEGATIVE
Cashew	NEGATIVE	Sunflower seeds	NEGATIVE	Brazil Nut	NEGATIVE
Rice	NEGATIVE	Pistachio	NEGATIVE	Macadamia Nut	NEGATIVE
Oatmeal	NEGATIVE	Poppy seed	NEGATIVE	Egg	NEGATIVE
Peanut	NEGATIVE	Pumpkin seed	NEGATIVE	Barley	NEGATIVE
Raw meat	INVALID	Soy (bean)	NEGATIVE	Lecithin	NEGATIVE
Rye	NEGATIVE	Wheat	NEGATIVE	Buckwheat	NEGATIVE
Chickpea	NEGATIVE	Hazelnut	NEGATIVE	Beans	NEGATIVE
Cornmeal	NEGATIVE	Cocoa	NEGATIVE	Sausages	NEGATIVE
Juice	NEGATIVE	Wine	NEGATIVE	Soy seed	NEGATIVE
Pasta	NEGATIVE	Salad dressing	NEGATIVE	Milk powder	POSITIVE
Raw milk	POSITIVE	UHT milk	POSITIVE		

3.3. Analysis of work surfaces

PROTEON MILK EXPRESS was tested on a stainless steel surface, commonly used in the food and catering industry. To do this, different amounts of skimmed milk powder (MoniQA), previously dissolved in solution, were dispensed on a surface, allowed to dry and then the test was carried out.

Table 3. Analysis of work surfaces

Skimmed milk powder (µg)	Milk proteins (µg)	β-lactoglobulin (µg)	Positives (%) to β-lactoglobulin
1.1	0.4	0.03	0
2.0	0.7	0.06	100
3.0	1.1	0.08	100
12.5	4.5	0.31	100

The results showed that up to 0.7 µg of milk protein can be detected with PROTEON MILK EXPRESS on any type of surface. According to the instructions of the FIAB and Food and Drink Europe, the size of the surfaces that must be analyzed is at least 100 cm2. Consequently, a positive result with PROTEON MILK EXPRESS indicates that there is at least 0.7 µg of milk protein on the analyzed surface.

4. CONVERSION FACTORS

Table 4. Conversion factors between milk and milk proteins (2).

Milk powder	Total proteins	β-lactoglobulin
1.0 ppm	0.35 ppm	0.03 ppm

5. BIBLIOGRAPHY

1. Appendix F: Guidelines for Standard Method Performance Requirements. Official Methods of Analysis (2016), AOAC INTERNATIONAL, Rockville, MD, USA (http://www.eoma.aoac.org/app_f.pdf).

2. Bobe et al. (2007) Composition of Milk Protein and Milk Fatty Acids Is Stable for Cows Differing in Genetic Merit for Milk Production. J Dairy Sci 90:3955-3960.