| VERSION 17

CAT.NUMBER: B1748

STORAGE: 2-8°C

BIO-SHIELD

COW CHEESE

ELISA TEST | In vitro analysis

for the detection and quantification of cow's milk in brine soft cheese

PROGNOSIS BIOTECH

This is an electronic version, please verify always the last one included in the kit.

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Use only the current version of Product Data Sheet enclosed with the kit.

Bio-Shield Cow Cheese, B1748 is an immunoassay method that detects the adulteration of sheep's or goat's brine soft cheese (like Feta) with cow's milk. The ELISA kit contains all reagents required for the immunoassay method. The ELISA test is adequate for 48 (standards are included). A spectrophotometer for microtiter ELISA plate is required.

- Sample preparation: extraction and dilution
- Test time (incubation time after samples and reagents preparation): 85min
- Shelf life: 12 months
- Storage: 2-8^{o}C

1. Description

Bio-Shield Cow Cheese is the only ELISA product in the market, especially designed to detect the composition of cow milk in mature soft cheese.

2. General Information

Unknown milk composition of cheese resulted by using adulterating or untracked milk in cheese manufacture results in a final product inferior to that expected by the consumer. Due to unknown milk mixtures used,changes occur in the final sensory characteristics and quality of cheese.Besides that , in certain Protected Designation of Origin (PDO) products like Feta, the use of cow milk is prohibited. In addition, Cow's milk allergy (CMA) is one of the most common food allergies. Milk can induce allergic reactions at infancy, this allergy is normally outgrown in the first year of life, however 15% of allergic children remain allergic, as a result cow milk should be declared as an ingredient on food labels.This certain Elisa kit can be used to detect and quantify the presence of cow milk, in mature soft cheese like Feta.

3. Principle of the Method

Cow IgG (immunoglobulin G) is naturally present in cow's milk and the presence of this milk in a milk sample is determined by the immunological detection of cow IgG. The wells of the microtiter strips are coated with very specific antibodies against cow IgG. The standard solutions and the solutions of the samples are added and if a specimen is an adulterated milk, the milk cow IgG will bind with the immobilized antibodies. All of the other ingredients that are not bond will be removed by washing. Then, the detection solution is added (peroxidaseconjugated antibody against cow IgG) and binds to cow IgG. The detection solution that have not reacted will be removed by washing. A chromogenic substrate is then added to the plate causing progressive development of a blue colored complex with the detection antibody. The color development is blocked after the addition of acid which converts the final solution from blue to yellow. The measurement is made photometrically at 450 nm and the intensity of the produced coloured complex is directly proportional to the concentration of cow IgG that are present in the samples and the standard solutions.

4. Reagents Provided

Bio-Shield Cow Cheese ELISA kit contains sufficient reagents and materials for 48 measurements (including standard tests).

Reagents (Store at 2-8ºC)	Quantity for 48 wells	State	Vial cap color
Single-Break Strip Plate	48 wells	Ready to use (precoated)	-
Standards 1-5 (0, 0.25, 1, 2, and 4% cow's milk in sheep's milk)	5 glass vials (each 1.5ml)	Ready to use	Black
Cow Cheese Detection Solution	1 plastic vial (6ml)	Ready to use	Green
Wash Buffer	1 plastic vial (50ml)	20X Concentrate (dilute in distilled water)	White
TMB Substrate	1 plastic vial (6ml)	Ready to use	Brown
Stop Solution	1 plastic vial (6ml)	Ready to use	White

5. Materials required but not provided

- Vortex mixer and Microtiter plate reader fitted with 450 nm filter.
- 100 and 1000µl adjustable single channel micropipettes with disposable tips (a repetitive pipette of 100µl is preferable for the steps of Detection Solution, TMB and Stop Solution).
- 50 - 300µl multi-channel micropipette with disposable tips and reservoirs.
- Distilled water, methanol, dichloromethane, n-hexane and pepsin.

6. Storage Instructions

Store kit reagents between 2 and 8°C (35 - 46°F). Do not freeze any components provided. Reseal the unused strips of the microtiter plate in the bag together **with the desiccant bag** provided and store at 2 - 8 °C. After use the remaining reagents should be returned to cold storage (2 - 8°C). Expiry of the kit and reagents is stated on the labels respectively and no quality guarantee is accepted after the expiration date. The expiry of the kit components can only be guaranteed if the components are stored properly as well as if the reagent is not contaminated by the first handling, in case of repeated use of one component. Because of the colorless chromogen substrate light sensitivity, avoid the exposure to direct light. Do not interchange individual reagents between kits of different lot numbers.

7. Safety and Precautions for use

- Avoid any skin contact with Stop Solution (15% H_3PO_4) and TMB (toxic). **Use gloves.** In case of contact, wash thoroughly with water.
- All reagents should be warmed in room temperature before use and covered when not in use. **Use a clean disposable plastic pipette tip for each reagent, in order to avoid cross contamination. When pipetting reagents, maintain a consistent order of addition from well-to-well. This will ensure equal incubation times for all wells.**
- Use a clean plastic container to prepare the wash buffer and all residual washing liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper into the well. Read the absorbance within 60 minutes after completion of the assay.

8. Indication of corruption of kit reagents

- The bluish coloration of the chromogen substrate before the ELISA test.
- A value of less than 0.7 absorbance units (ABS 450nm) for the Standard 1 (St1).

9. Method Procedure

9.1 Determine the desirable number of samples to be tested and prepare a number of tubes (10-15ml) respectively. Mark the tubes appropriately.

9.2 Dilute the 20X solution concentrate 20 fold with distilled water to give a **1X** working solution.

Preparation of Wash Buffer 1X: In case of the occurrence of crystals in the Wash Buffer, the warming by gentle dismantling (using hands) of the crystals is needed. Pour entire content of the solution concentrate (50ml) into a clean 1000ml graduated cylinder, rinse the vial with distilled or deionised water and pour the content again into the cylinder and fill to a final volume of 1000ml with distilled or deionised water. Mix gently to avoid foaming, transferring the final solution from cylinder to a clean bottle and back two times. The clean bottle with **1X Wash Buffer** working solution can be left out of the refrigerator during the method procedure and subsequent be stored 2 - 8°C for one month.

9.3 Assay Design: Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for standards. Considering that each sample and standard can be tested in single or in duplicate, create a layout. **NOTE:** If the number of wells is more than 32 (four strips), a repetitive pipette or multichannel pipette is necessary.

CAUTION: Use the standards positions in duplicate as the **Example plate** layout below **NECESSARY** and note positions of samples that can be set to all remaining empty wells of layout in duplicate.

	1	2	3	4	5	6	7	8	9	10	11	12
A	St1	St1										
B	St2	St2										
C	St3	St3										
D	St4	St4										
E	St5	St5										
F												
G												
H												

Example plate layout (example for a 5 point standard curve)

9.4 Weigh 2gr of each dry cheese sample and place the samples to the marked tubes respectively.

9.5 **Cheese extraction.** Add 6mL of **Wash Buffer 1X** to each tube (containing the sample of 2gr cheese). Close each tube with the cap and shake it for 10-15 seconds. Remove each cap and homogenized gently the sample using a glass rod for 2 - 4 minutes. Then, filter each sample using Whatman No1 filter paper and collect 2 - 2.5mL of filtrate. Each filtrate can be stored at -20°C for future use. **NOTE:** In order to reduce the time needed, centrifuge each sample at 3000g for 5 minutes and then filter the supernatant.

9.6 Preparation of Samples: The samples should be stored in a cool place. Stir well the cheese samples before the dilution, dilute **1:16** with **Wash Buffer 1X** working solution and agitate well the cheese dilutions (vortex):

100µl cheese extract + **1500µl** Wash Buffer 1X

9.7 Bring all reagents to room temperature (19-24°C) before use. Remove the **standards** (Standard 1-5) and the **appropriate number of wells** into the holder of microwells for the standards and the samples to be worked in duplicate. Place the wells into the holder of microwells and immidiately reseal the unused strips of the microtiter plate in the bag together with the desiccant bag provided.

9.8 Add 100 µl per well of each standard **(Standard 1 - 5)** or prepared sample (see 10.4) in duplicate. Cover the microwells with the sealing film, shake the microplate manually for 30 seconds and incubate at room temperature for **45min**.

9.9 **Washes:** Remove the sealing film and wash the plate as follows: Aspirate the liquid from each well into the sink and tap the holder of microwells upside down strongly (four times in a row) on an absorbent paper to insure the complete removal of liquid from the wells. Dispense **300µl** of **Wash Buffer 1X** (see 9.2) into each well with wash bottle or multichannel micropipette using the proper reagent reservoir and shaking the plate manually for a few seconds. Repeat this process for another three times **(total 4 times). CAUTION:** It is important to not allow microwells to dry between working steps.

9.10 Aspirate the liquid as described above and add **100µl** of **Cow Cheese Detection Solution** to each well. If the number of wells is more than 32 (four strips), a repetitive pipette or multichannel pipette is necessary (pour 1 ml of Detection Solution in a reservoir per 8 wells). Cover the microwells with the sealing film, shake the plate manually for a 30 seconds and incubate at room temperature for **30min**.

9.11 Remove the sealing film and wash the plate as the wash **step 9.9**.

9.12 Aspirate the liquid as described above and add **100 µl** per well of **TMB Substrate** (pour 1ml per 8 wells in a reservoir). Cover the microwells with the sealing film, shaking the plate manually for a few seconds and incubate in the dark at room temperature for **10min**.

9.13 Remove the sealing film and add **100 µl** per well of the **Stop Solution** to each well (pour 1ml per 8 wells in a reservoir). Mix gently by shaking manually.

9.14 **Measure the absorbance at 450 nm**. Read the absorbance value of each well (immediately after the step 10.11 on a spectrophotometer using 450 nm as the primary wavelength and optionally 620 nm as the reference wave length (610 nm to 650 nm is acceptable).

10. Data Analysis

- **Automatically**

An assigned software, the **Prognosis-Data-Reader,** is available for free (contact:info@prognosis-biotech.com) download in order to evaluate the Bio-Shield Cow Cheese ELISA kit. The evaluation is carried out by a simple transfer of data values after the measurement.

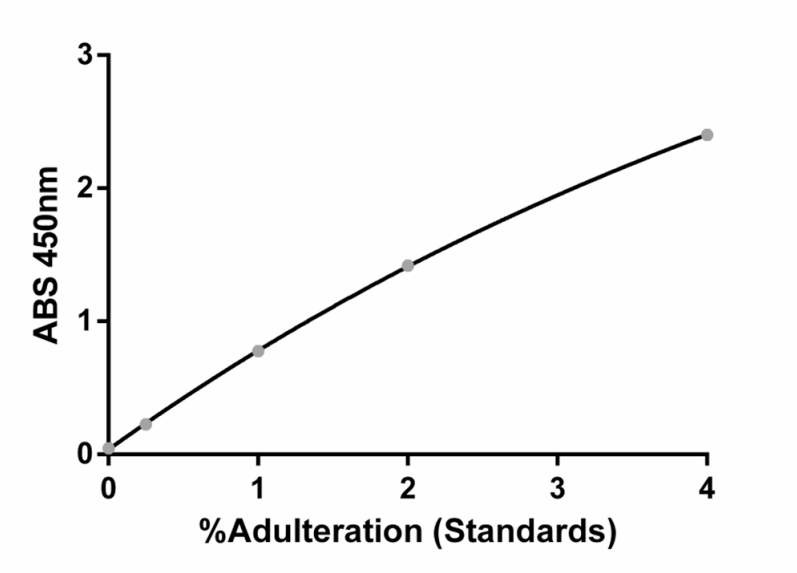
- **Manually**

Calculate the average absorbance values for each set of duplicate standards and samples. Ideally duplicates should be within 10% of the mean. Use the following calculation:

$$\frac{\text{Standard or sample absorbance}}{\text{Standard 5 absorbance}} \times 100 = \%\ \text{Binding}$$

The adulteration (%) in each sample is determined by extrapolating OD values against adulterations of standard solutions using a fifth order polynomial standard curve.

11. Example of Standard Curve (0 – 4%)



12. Immunoassay Specification

12.1 General Specification

- B/Bmax 1% = 25-45%
- Each standards duplicates mean CV ≤ 6%
- Coefficient of Variation (CV) of result at 1% = 5.5% (n=16)

12.2 LOD - LOQ - Recovery

LOD	0.04%
LOQ	0.15%
Recovery	100%

12.3 Specificity

Immunoglobulins	Cross Reactivity of the antibody (%)
Bovine IgG	100
Sheep IgG	<0.01
Goat IgG	<0.01
Buffalo IgG	3.2

13. Method Summary

Total procedure time (after samples and reagents preparation): 85 min.

Cheese extraction and filtrate dilution

⇩

Add 100 µl of each standard and sample in microplate and incubate 45 min at room temperature

⇩

Wash four times

⇩

Add 100 µl of ready-to-use Detection Solution and incubate 30 min at room temperature

⇩

Wash four times

⇩

Add 100 µl of ready-to-use TMB and let the color develop for 10 min in the dark at room temperature

⇩

Add 100 µl Stop Solution and read absorbance at 450 nm within 60 min

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This is an electronic version, please verify always the last one included in the kit.

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