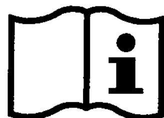
Biogradetech



Product information

beta-Lactoglobulin ELISA Kit

REF **BGT-KET-366**

96T

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Sensitivity (ß-Lactoglobulin)	1.5 ppb
Recovery	70-107%
Incubation Time	60 min

1. GENERAL INFORMATION

Bovine milk belongs to the most important allergenic food ingredients especially for children. Already very low amounts of bovine milk can cause allergic reactions, which may lead to anaphylactic shock in severe cases. Because of this, milk allergic persons must strictly avoid the consumption of milk or milk containing food. In particular the presence of hidden milk proteins such as in sausage, cookies, convenience food or beverages represent a critical problem for milk allergic persons. According to EU Directive 2003/89/EG the addition of bovine milk has to be labeled. For the detection of bovine milk in foodstuffs sensitive detection systems are required. Approximately 20% of bovine milk proteins are whey proteins. The main fraction of whey proteins consists of the heat-stable allergen ß-lactoglobulin. The **beta-Lactoglobulin ELISA** represents a highly sensitive detection system and is particularly capable of the identification and quantification of bovine milk residues in cookies, cereals, sausage, orange juice, wine, soy products and chocolate.

2. PRINCIPLE OF THE TEST

The **beta-Lactoglobulin ELISA** quantitative test is based on the principle of the enzyme linked immunosorbent assay. An antibody directed against ß-lactoglobulin is bound on the surface of a microtiter plate. ß-Lactoglobulin containing samples or standards are given into the wells of the microtiter plate. After 20 minutes incubation at room temperature, the wells are washed with diluted washing solution to remove unbound material. A peroxidase conjugated second antibody directed against ß-lactoglobulin is given into the wells and after 20 minutes of incubation the plate is washed again. A substrate solution is added and incubated for 20 minutes, resulting in the development of a blue colour. The colour development is inhibited by the addition of a stop solution, and the colour turns yellow. The yellow colour is measured photometrically at 450 nm. The concentration of ß-lactoglobulin is directly proportional to the colour intensity of the test sample.

3. PRECAUTIONS

Full compliance of the following good laboratory practices (GLP) will determine the reliability of the results:

1. Prior to beginning the assay procedure, bring all reagents to room temperature (20-25°C).
2. All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
3. Once the assay has been started, all subsequent steps should be completed without interruption and within the recommended time limits.
4. Replace caps in all the reagents immediately after use. Do not interchange vial stoppers.
5. Use a separate disposable tip for each specimen to prevent cross-contamination.
6. All specimens and standards should be run at the same time, so that all conditions of testing are the same.
7. Do not mix components from different batches.
8. Do not use reagents after expiration date.
9. Check both precision and accuracy of the laboratory equipment used during the procedure (micropipettes, ELISA reader etc.).

4. HEALTH AND SAFETY INSTRUCTIONS

1. Do not smoke or eat or drink or pipet by mouth in the laboratory.
2. Wear disposable gloves whenever handling patient specimens.
3. Avoid contact of substrate and stop solution with skin and mucosa (possible irritation, burn or toxicity hazard). In case of contact, rinse the affected zone with plenty of water.
4. Handling and disposal of chemical products must be done according to good laboratory practices (GLP).

5. REAGENTS

The kit contains reagents for 96 determinations. They have to be stored at 2-8°C. Expiry data are found on the labels of the bottles and the outer package.

1. **SORB** **MT** Microtiter plate consisting of 12 strips with 8 breakable wells each, coated with anti-ß-lacto-globulin antibodies.
2. **CAL** **1** **–** **5** ß-Lactoglobulin Standards (0, 10, 40, 100, 400 ppb of ß-lactoglobulin): 5 vials with 2.0 mL each, dyed red, ready-to-use.
3. **ENZ** **CONJ** Conjugate (anti-ß-lactoglobulin-peroxidase): 15 mL, dyed red, ready-to-use.
4. **SUB** **TMB** Substrate Solution (TMB): 15 mL, ready-to-use.
5. **STOP** **SOLN** Stop Solution (0.5 M H_2SO_4): 15 mL, ready-to-use.
6. **SAM** **DIL** **10x** Extraction and sample dilution buffer (Tris): 2 x 120 mL as 10x concentrate, dyed red. Dilute 1+9 with distilled water. Stored at 4°C the diluted buffer is stable for at least one week. If during the cold storage crystals precipitate, the concentrate should be warmed up to 37°C for 15 minutes.
7. **WASH** **SOLN** **10x** Washing Solution (PBS + Tween 20): 60 mL as 10x concentrate. Dilute 1+9 with distilled water. Stored at 4°C the diluted buffer is stable for at least 4 weeks. If during the cold storage crystals precipitate, the concentrate should be warmed up to 37°C for 15 minutes.
8. Instruction Manual.

6. ADDITIONAL INSTRUMENTATION AND REAGENTS (NOT PROVIDED)

Instrumentation

- 100 - 1000 µL micropipettes
- Analytical balance
- Mortar, mixer
- Water bath
- Centrifuge
- ELISA reader (450 nm)
- Plastic bag to store unused microtiter strips

Reagents

- double distilled water

7. SAMPLE PREPARATION

Due to high risk of cross-contamination all applied instruments like applicator, mortar, glass vials etc. have to be **cleaned thoroughly** before and after each sample. To identify possible cross-contamination caused by previous extractions it is strongly recommended to note the sequence of the extractions.

The following sample preparation should be applied for solid samples:

1. To maximize homogeneity and representativeness of the sample drawing, a minimum of 5 g sample should be pulverized finely in a mortar, impact mill etc.
2. 1 g of the homogenized mixture is suspended in 20 mL of **pre-diluted** extraction buffer. Afterwards the suspension is incubated for 15 min in a preheated water bath at 60°C. To ensure good homogeneity, the samples should be shaken every two minutes.
3. The samples are centrifuged for 10 minutes at 2000 g. If it is not possible to separate the supernatant from the precipitate completely, the suspension should be filtrated if necessary.
4. 100 µL of particle-free solution are applied per well. If the results of a sample are out of the measuring range, further dilution with the **pre-diluted** extraction and sample dilution buffer is necessary. The additional dilution has to be considered when calculating the concentration.

The following sample preparation should be applied for liquid samples:
1 mL of liquid sample is diluted in 19 mL of **pre-diluted** extraction buffer. Afterwards the suspension is incubated for 15 min in a preheated water bath at 60°C. To ensure good homogeneity, the samples should be shaken every two minutes. The process is continued at point 3 of solid sample extraction process.

8. PROCEDURE

The washing solution is supplied as 10x concentrate and has to be **diluted** 1+9 with double distilled water before use. In any case the **ready-to-use** standards provided should be determined twofold. When samples in great quantities are determined, the standards should be pipetted once before the samples and once after the samples. For final interpretation the arithmetic mean is used for calculation. In consideration of GLP and quality control requirements a duplicate measurement of samples is recommended.

The procedure is according to the following scheme:
1. Prepare samples as described above.
2. Pipet 100 µL **ready-to-use** standards or prepared samples in duplicate into the appropriate wells of the microtiter plate.
3. Incubate for 20 minutes at room temperature.
4. Wash the plate three times as follows: Discard the contents of the wells (dump or aspirate). Pipet 300 µL of diluted washing solution into each well. After the third repetition empty the wells again and remove residual liquid by striking the plate against a paper towel. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbencies.
5. Pipet 100 µL of conjugate (anti-ß-lactoglobulin-peroxidase) into each well.
6. Incubate for 20 minutes at room temperature.
7. Wash the plate as outlined in 4.
8. Pipet 100 µL of substrate solution into each well.
9. Allow the reaction to develop in the dark (e.g. cupboard or drawer; the chromogen is light-sensitive) for 20 minutes at room temperature.
10. Stop enzyme reaction by adding 100 µL of stop solution (0.5 M H_2SO_4) into each well. The blue colour will turn yellow upon addition.
11. After thorough mixing, measure absorbance at 450 nm (reference wavelength 620 nm), using an ELISA reader. The colour is stable for 30 minutes.

9. CALCULATION OF RESULTS

The ready-to-use standards are prepared for a direct determination of sample concentrations. The dilution of samples in the extraction process as described in the above stated sample preparation procedure is already considered. Additional dilution due to high sample concentration has to be accounted for.

1. Calculate the average optical density (OD 450 nm) for each set of reference standards or samples.
2. Construct a standard curve by plotting the mean optical density obtained for each reference standard against its concentration in ppb on semi-log graph paper with the optical density on the vertical (y) axis and the concentration on the horizontal (x) axis. Alternatively the evaluation can be carried out by software. In this case the 4-parameter method should be preferred.
3. Using the mean optical density value for each sample, determine the corresponding concentration of ß-lactoglobulin in ppb from the standard curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.

10. TYPICAL STANDARD VALUES

The following table contains an example for a typical standard curve. The binding is calculated as percent of the absorption of the 400 ppb standard. These values are only an example and should not be used instead of the standard curve which has to be measured in each new test.

ß-Lactoglobulin (ppb)	% binding of 400 ppb
400	100
100	61
40	36
10	15
0	5

11. PERFORMANCE

Sensitivity

The limit of detection (LOD) of the**Beta-Lactoglobulin ELISA test** is 1.5 ppb. Validation experiments with milk (products) showed that this corresponds approximately to 0.6 ppm unskimmed milk, 0.1 ppm non fat milk powder (NIST RM1549) and 0.5 ppm whole milk powder (NIST RM8435). The limit of quantification (LOQ) of the **Beta-Lactoglobulin ELISA test** is 10 ppb. Validation experiments with milk (products) showed that this corresponds approximately to 3.7 ppm unskimmed milk, 0.6 ppm non fat milk powder (NIST RM1549) and 3.2 ppm whole milk powder (NIST RM8435). Due to the variety of sample matrices and their influence on the blank, results less than the LOQ should be treated as negative.

Cross-reactivity

For the following foods no cross-reactivity could be detected:

Adzuki bean	Cayenne	Duck	Kiwi	Pepper, black	Shrimps
Almond	Celery	Egg	Lamb	Pine seed	Soy flour
Apricot	Cherry	Fennel	Leek	Pistachio	Soy lecithin
Barley	Chestnut	Fenugreek	Lentil	Plum	Split pea
Bean, white	Chia seeds	Flaxseed	Lupin	Poppy	Sunflower seed
Beef	Chicken	Garden cress	Macadamia	Pork	Thyme
Beef, cooked	Chickpea	Garlic, fresh	Mustard, yellow	Potato	Tofu
Bovine gelatin	Chili	Garlic, granulated	Nutmeg	Prawn cooked	Tomato
Brazil nut	Cinnamon	Ginger, fresh	Oats	Prawn, raw	Turkey
Buckwheat	Clove	Ginger, ground	Onion	Pumpkin seed	Turmeric
Cabbage, white	Cocoa	Gliadin	Orange	Radish	Walnut
Caraway	Coconut	Guar gum	Paprika	Rapeseed	Wheat
Cardamom	Cod	Gum arabic	Pea	Rice	Wine, red
Carob gum	Corn	Hazelnut	Peach	Rye	Wine, white
Carrot	Cumin	Horseradish	Peanut	Saccharose	
Cashew	Dill	Kidney bean	Pecan	Sesame	

For the following commodities of the table above the results were between 0.5*LOQ and LOQ of the kit. So, it cannot be completely excluded that these matrices may provide values above the LOQ in specific cases:

Horseradish	Radish	Sunflower seed

The following cross reactions were determined:

Ewe's milk	< 0.2%
Goat's milk	< 0.002%
Casein	< 0.02%
Bovine serum albumin	0.0000012%

Precision

Intra-assay Precision	7%
Inter-assay Precision	9 - 12%

Linearity

The serial dilution of spiked samples (cookies, cereals, chocolate, sausage, soy milk, orange juice and white wine) resulted in a dilution linearity of 72% - 127%.

Recovery

Mean recovery was determined by spiking samples with different amounts of ß-lactoglobulin:

Empfindlichkeit (ß-Lactoglobulin) 1,5 ppb
Wiederfindung 70 – 107%
Inkubationszeit 60 min

Cookies	88%
Cereals	94%
Chocolate	86%
Sausage	107%
Soy milk	70%
Orange juice	98%