BOVINE CASEIN

Immunoperoxidase Assay for Determination of Bovine Casein in foods

Directions for Use

Version 2 4.0 -- 6

I. INTENDED USE

The Bovine Casein test kits are a highly sensitive two-site enzyme linked immunoassay (ELISA) for measuring Bovine Casein in foods.

II. PRINCIPLE OF THE ASSAY

The principle of the double antibody sandwich ELISA is represented in Figure 1. In this assay the Bovine Casein present in sample reacts with the anti-Bovine Casein antibodies which have been adsorbed to the surface of polystyrene microtitre wells. After the removal of unbound proteins by washing, anti-Bovine Casein antibodies conjugated with horseradish peroxidase (HRP), are added. These enzyme-labeled antibodies form complexes with the previously bound Bovine Casein. Following another washing step, the enzyme bound to the immunosorbent is assayed by the addition of a chromogenic substrate, 3,3',5,5'-tetramethylbenzidine (TMB). The quantity of bound enzyme varies directly with the concentration of Bovine Casein in the sample tested; thus, the absorbance, at 450 nm, is a measure of the concentration of Bovine Casein in the test sample. The quantity of Bovine Casein in the test sample can be interpolated from the standard curve constructed from the standards, and corrected for sample dilution.

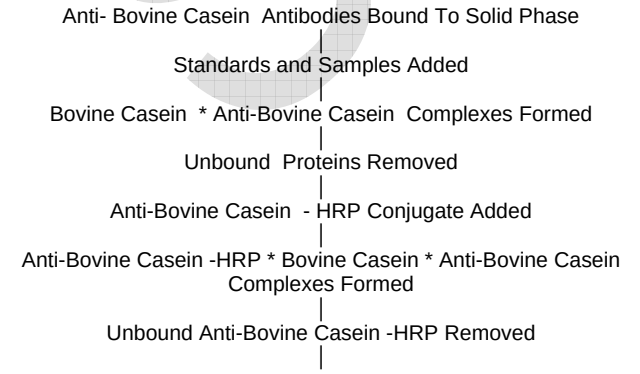

Figure 1.

III. REAGENTS (Quantities sufficient for 96 determinations)

1. DILUENT CONCENTRATE (Running Buffer)
One bottle containing 50 ml of a 5X concentrated diluent running buffer.

2. WASH SOLUTION CONCENTRATE
One bottle containing 50 ml of a 20X concentrated wash solution.

3. EXTRACTION BUFFER
One bottle of 2X Extraction Buffer containing 120 mL.

WARNING: Avoid contact with skin.

4. ENZYME-ANTIBODY CONJUGATE 1X
One vial containing 11 mL of affinity purified anti-Bovine Casein antibody conjugated with horseradish peroxidase in a stabilizing buffer.

5. CHROMOGEN-SUBSTRATE SOLUTION
One vial containing 12 mL of 3,3',5,5'-tetramethybenzidine (TMB) and hydrogen peroxide in citric acid buffer at pH 3.3.

6. STOP SOLUTION
One vial containing 12 ml 0.3 M sulfuric acid.

WARNING: Avoid contact with skin.

7. ANTI-BOVINE CASEIN ELISA MICRO PLATE
Twelve removable eight (8) well micro well strips in well holder frame. Each well is coated with affinity purified anti-Bovine Casein.

8. BOVINE CASEIN STANDARDS
Four vials containing 2.0mL each of pre-diluted Bovine Casein standards at different concentrations.

FOR IN VITRO USE ONLY

IV. REAGENT PREPARATION

1. DILUENT CONCENTRATE
The Diluent Solution supplied is a 5X Concentrate and must be diluted 1/5 (1 part buffer concentrate, 4 parts dH2O) with distilled or deionized water.

2. WASH SOLUTION CONCENTRATE
The Wash Solution supplied is a 20X Concentrate and must be diluted 1/20 with distilled or deionized water (1 part buffer concentrate, 19 part dH2O). Crystal formation in the concentrate is not uncommon when storage temperatures are low. Warming of the concentrate to 30-35°C before dilution can dissolve crystals.

3. EXTRACTION BUFFER
The Extract Buffer supplied is a 2X concentrate and must be diluted 1/2 (1 part buffer concentrate, 1 parts dH2O) with distilled or deionized water.

The diluted buffer should be warmed to 50-60°C before use.

4. ENZYME-ANTIBODY CONJUGATE
The 1X conjugate solution is ready to use as supplied.

5. CHROMOGEN-SUBSTRATE SOLUTION
Ready to use as supplied.

6. STOP SOLUTION
Ready to use as supplied.

7. ANTI-BOVINE CASEIN ELISA MICRO PLATE
Ready to use as supplied. Unseal Microtiter Pouch and remove plate from pouch. Remove all strips and wells that will not be used in the assay and place back in pouch and re-seal. Take clear plastic tape and cover tops of strips to avoid particulates from contaminating wells.

8. BOVINE CASEIN STANDARDS
Ready to use as supplied.

V. STORAGE AND STABILITY

The expiration date for the package is stated on the box label.

1. DILUENT
The 5X Diluent Concentrate is stable until the expiration date. The 1X working solution is stable for at least one week from the date of preparation. Both solutions should be stored at 4-8°C.

2. WASH SOLUTION
The 20X Wash Solution Concentrate is stable until the expiration date. The 1X working solution is stable for at least one week from the date of preparation. Both solutions can be stored at room temperature (16-25°C) or at 4-8°C.

3. EXTRACTION BUFFER
The Extraction Buffer, diluted and undiluted, should be stored at 4-8°C and is stable until the expiration date.

4. ENZYME-ANTIBODY CONJUGATE
Undiluted horseradish peroxidase anti-Bovine Casein conjugate should be stored at 4-8°C until the expiration date.

5. CHROMOGEN-SUBSTRATE SOLUTION
The Substrate Solution should be stored at 4-8°C and is stable until the expiration date.

6. STOP SOLUTION
The Stop Solution should be stored at 4-8°C and is stable until the expiration date.

7. ANTI-BOVINE CASEIN ELISA MICRO PLATE
Anti- BOVINE CASEIN coated wells are stable until the expiration date, and should be stored at 4-8°C in the sealed foil pouch with desiccant pack.

8. Bovine CASEIN STANDARDS
Stable until expiration date and should be stored at 4C. Do not freeze.

INDICATIONS OF INSTABILITY
If the test is performing correctly, the results observed with the standard solutions should be within 20 % of the expected values.

VI. SAMPLE PREPARATION

Bring all reagents to room temperature before use.

1. Food samples should be extracted using the preheated diluted extraction buffer. A homogeneous mixture is necessary for proper extraction. Solid food samples must be blended/grounded to a fine consistency to ensure proper extraction.

2. For solid food samples, weigh out 1 g of a well blended/ground sample and add 9 ml of preheated diluted extraction buffer. Vortex to mix. The ratio is 1 part sample to 9 volumes of buffer.

3. For liquid samples, a 1/10 dilution is used with preheated diluted extraction buffer. Add 9 ml of extraction buffer to 1 ml of homogenous liquid sample. Vortex to mix.

4. CIP (Clean in Place) Solutions can be run with a simple 1/5 dilution in running buffer (see V. 1 Above)

5. Then shake samples in 50-60°C shaking water bath for ~30 minutes. Or leave samples in water bath for 30 minutes and shake/mix for 1 minute every 5 minutes.

6. After extraction, centrifuge samples to pellet particulates or allow them to sit for 5min in test tube rack.

7. Take 100ul from middle layer and add it to 900ul of diluted running buffer. Vortex or mix well. Note: The total dilution of the sample is now 1/100.

8. Known interfering substances

Azide and thimerosal at concentrations higher than 0.1% inhibits the enzyme reaction.

VII. MATERIAL PROVIDED
See "REAGENTS"

MATERIALS REQUIRED
BUT NOT PROVIDED

- Precision pipette (2 μL to 200 μL) for making and dispensing dilutions
- Test tubes
- Microtitre washer/aspirator
- Distilled or Deionized H_2O
- Microtitre Plate reader
- Assorted glassware for the preparation of reagents and buffer solutions
- Timer
- Vortex mixer
- Shaking water bath

VIII. PROCEDURE

Bring all reagents to room temperature before use.

1. Add 100 μL of Diluent to each of the wells in 1A & 2A. These will serve for an evaluation of the background associated with the assay.

2. Pipette 100 μL of
   1X Diluent (0.0 ng/ml) in duplicate
   Standard 1 (4.44 ng/ml) in duplicate
   Standard 2 (13.33 ng/ml) in duplicate
   Standard 3 (40 ng/ml) in duplicate
   Standard 4 (120 ng/ml) in duplicate

2. Pipette 100 μL of sample (in duplicate) into pre designated wells.

4. Incubate the micro titer plate at room temperature for fifteen (15 ± 2) minutes. Keep plate level during incubation.

5. Following incubation, aspirate the contents of the wells.

6. Completely fill each well with appropriately diluted Wash Solution and aspirate. Repeat three times, for a total of four washes. If washing manually; completely fill wells with wash buffer, invert the plate and pour/shake out the contents in a waste container. Follow this by sharply striking the wells on absorbent paper to remove residual buffer. Repeat 4 times for a total of five washes.

7. Pipette 100 μL of appropriately diluted Enzyme-Antibody Conjugate to each well. Incubate at room temperature for ten (10 ± 2) minutes.

8. Wash and blot the wells as described in Step 5/6.

9. Pipette 100 μL of TMB Substrate Solution into each well.

10. Incubate in the dark at room temperature for precisely ten (10) minutes.

11. After ten (10) minutes, add 100 μL of Stop Solution to each well.

12. Determine the absorbance (450 nm) of the contents of each well. Calibrate the plate reader to air.

IX. STABILITY OF THE FINAL REACTION MIXTURE

The absorbance of the final reaction mixture can be measured up to 2 hours after the addition of the Stop Solution. However, good laboratory practice dictates that the measurement be made as soon as possible.

X. RESULTS

1. Subtract the average background value from the test values for each sample.

2. Using the results observed for the standards construct a Standard Curve. The appropriate curve fit is that of a four-parameter logistics curve. A second order polynomial (quadratic) or other curve fits may also be used.

3. Calculate the sample concentrations off the standard curve, result unit is in ng/ml (ppb). Then, multiply by sample dilution factor to get the concentration of original samples. For example, if total dilution of the sample is 1/100, and sample concentration off the standard curve is 200ng/ml (ppb), the final sample concentration is 200ng/ml x 100 = 20,000 ng/ml (ppb) which is 20ug/ml (ppm).

XI. QUALITY CONTROL

In accord with good laboratory practice, the Assays for specific Bovine Casein require meticulous quality control. Each laboratory should use routine quality control procedures to establish inter- and intra-assay precision and performance characteristics.

XII. LIMITATION OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the information contained in the package insert instructions and with adherence to good laboratory practice.

2. Factors that might affect the performance of the assay include proper instrument function, cleanliness of glassware, quality of distilled or deionized water, washing thoroughly and accuracy of reagent and sample pipettings.

Manufactured by:

Immunology Consultants Laboratory, Inc.
141 N. Elliott Rd | Newberg, OR 97132 | USA
Phone (503) 538-5869 | Fax (503) 554-8510