Bovine Chlamydia Pneumoniae (CP) Antibody ELISA Kit

Cat# BGT-KET-01000

Name	**Bovine Chlamydia Pneumoniae Antibody ELISA Kit**
Full name	**Bovine Chlamydia Pneumoniae Antibody ELISA Test Kit**
Category Name	Infection Disease kits
Test	96
Method	ELISA method: Enzyme Linked Immunosorbent Assay
Principle	ELISA principle- Peroxidase conjugated
Detection Range	Qualitative ELISA assay - Positive, Negative
Sample	5-10 µl serum
Specificity	98.1%
Sensitivity	99.3%
Total Time	~ 100 min
Shelf Life	12 Months from the manufacturing date

INTENDED USE

The Bovine Chlamydia Pneumoniae Antibody Enzyme-Linked Immunosorbent Assay (ELISA) is intended for the detection of antibodies in bovine serum to Chlamydia Pneumoniae antigen.

SUMMARY AND EXPLANATION

Chlamydia pneumoniae is a species of Chlamydia, an obligate intracellular bacterium that infects humans and is a major cause of pneumonia. Chlamydia pneumoniae has a complex life cycle and must infect another cell to reproduce; thus, it is classified as an obligate intracellular pathogen. The full genome sequence for C. pneumoniae was published in 1999. It also infects and causes disease in koalas, emerald tree boas (Corallus caninus), iguanas, chameleons, frogs, and turtles. Chlamydia pneumoniae is a common cause of pneumonia around the world; it is

typically acquired by otherwise-healthy people and is a form of community-acquired pneumonia. Its treatment and diagnosis are different from historically recognized causes, such as Streptococcus pneumoniae.

C. pneumoniae infection triggers acute wheezing, if it becomes chronic then it is diagnosed as asthma. These observations suggest that acute C. pneumoniae infection is capable of causing protean manifestations of chronic respiratory illness which lead to asthma. A recent case series of 101 adults with asthma reported that macrolides (mostly azithromycin) and tetracyclines, either separately or in combination, appeared to be dramatically efficacious in a subgroup of "difficult-to-treat" (i.e., not necessarily refractory to high-dose inhaled corticosteroids but who did not take them) patients with severe asthma, many of whom also had the "overlap syndrome" (asthma and COPD). Randomized, controlled trials that include these types of asthma patients are needed.

There is currently no vaccine to protect against Chlamydia pneumoniae. Identification of immunogenic antigens is critical for the construction of an efficacious subunit vaccine against C. pneumoniae infections. Additionally, there is a general shortage worldwide of facilities that can identify/diagnose Chlamydia pneumoniae.

TEST PRINCIPLE

This Chlamydia Pneumoniae test is an Enzyme-Linked Immunosorbent Assay to detect antibodies to Chlamydia Pneumoniae antigen. Native Chlamydia Pneumoniae antigen is attached to a solid phase microassay well. Enzyme-Linked Immunosorbent Assays (ELISA) rely on the ability of biological materials (i.e., antigens) to adsorb to plastic surfaces such as polystyrene (solid phase). When antigens bound to the solid phase are brought into contact with a bovine's serum, antigen specific antibody, if present, will bind to the antigen on the solid phase forming antigen-antibody complexes. Excess antibody is removed by washing. This is followed by the addition of goat anti-bovine IgG conjugated with horseradish peroxidase which then binds to the antibody-antigen complexes. The excess conjugate is removed by washing, followed by the addition of Chromogen/Substrate, tetramethylbenzidine (TMB). If specific antibody to the antigen is present in the bovine's serum, a blue color develops. When the enzymatic reaction is stopped with 1N H_2SO_4, the contents of the wells turn yellow. The color, which is indicative of the concentration of antibody in the serum, can be read on a suitable spectrophotometer or ELISA microwell plate reader.

SPECIMEN COLLECTION AND PREPARATION

1. Handle all blood and serum as if capable of transmitting infectious agents.
2. Optimal performance of the ELISA kit depends upon the use of fresh serum samples (clear, non-hemolyzed, nonlipemic, non-icteric). A minimum volume of 50 µL is recommended, in case repeat testing is required. Specimens should be collected aseptically by venipuncture. Early separation from the clot prevents hemolysis of serum.
3. Store serum between 2 and 8 ºC if testing will take place within five days. If specimens are to be kept for longer periods, store at -20 to -70 ºC in a non-defrosting freezer. Do not use a frost free freezer because it may allow the specimens to go through freeze-thaw cycles and

degrade antibody. Samples that are improperly stored or are subjected to multiple freeze-thaw cycles may yield erroneous results.
4. Serum containing visible particulate matter can be spun down utilizing slow speed centrifugation.
5. Do not use heat inactivated serum.
6. The NCCLS provides recommendations for storing blood specimens (Approved Standard - Procedures for the Handling and Processing of Blood Specimens, H18-A. 1990).12

MATERIALS AND COMPONENTS

The EIA Chlamydia Pneumoniae antigen antibody Test Kit contains supplies for 96 Test. Each kit contains the following components in sufficient quantities to perform the number of tests indicated on the package label

Materials provided with the test kits

1. **Purified Chlamydia Pneumoniae antigen coated microassay plate**: 96 wells, configured in twelve 1x8 strips, stored in a foil pouch with desiccant. (96T: one plate)

2. **Serum Diluent**: Ready to use. Contains proclin (0.1%) as a preservative. (96T: one bottle, 2 x 12 mL)

3. **Antibody Diluent:** Ready to use. Contains proclin (0.1%) as a preservative. (96T: one bottle, 1 x 12 mL)

4. **Positive Control**: bovine serum or defibrinated plasma. Sodium azide (< 0.1%) and pen/strep (0.01%) added as preservatives, with established range printed on vial label. The Positive Control is utilized to control the positive range of the assay. (96T: 62.5 x one vial, dilute to 0.5 mL with serum diluent to 1x)

5. **Negative Control:** bovine serum or defibrinated plasma. Sodium azide (< 0.1%) and pen/strep (0.01%) added as preservatives, with established range printed on vial label. The Negative Control is utilized to control the negative range of the assay. (96T: one vial, 0.5 mL, 1 x)

6. **Horseradish-peroxidase (HRP) Conjugate**: Goat anti-bovine IgG and IgM, containing proclin (0.1%) and gentamicin as preservatives. (96T: one tube, 5 µl, 10,000 x , needs to be diluted to 1x with Antibody Diluent before use)

7. **Wash Buffer (20X concentrate):** Dilute 1 part concentrate + 19 parts deionized or distilled water. Contains TBS, Tween-20 and proclin (0.1%) as a preservative. (96T: one bottle, 25 mL)

8. **Chromogen Solution I:** H_2O_2, ready to use. The reagent should remain closed when not in use. If allowed to evaporate, a precipitate may form in the reagent wells. (96T: one bottle, 6 mL)

9. **Chromogen Solution II:** Tetramethylbenzidine (TMB), ready to use. The reagent should remain closed when not in use. If allowed to evaporate, a precipitate may form in the reagent wells. (96T: one bottle, 6 mL), mix with equal volume of H_2O_2 before use

10. **Stop Solution:** Ready to use, contains a 1N H_2SO_4 solution. (96T: one bottle, 6 mL)

Materials required but not provided

1. Wash bottle, automated or semi-automated microwell plate washing system.
2. Micropipettes, including multichannel, capable of accurately delivering 10-200 µL volumes (less than 3% CV).
3. One liter graduated cylinder.
4. Paper towels.
5. Test tube for serum dilution.
6. Reagent reservoirs for multichannel pipettes.
7. Pipette tips.
8. Distilled or deionized water (dH20).
9. Timer capable of measuring to an accuracy of +/- 1 second (0 - 60 minutes).
10. Disposal basins and 0.5% sodium hypochlorite (50 mL bleach in 950 mL dH20).
11. Single or dual wavelength microplate reader with 450 nm filter. If dual wavelength is used, set the reference filter to 600-650 nm. Read the Operator's Manual or contact the instrument manufacturer to establish linearity performance specifications of the reader.

REAGENT PREPARATION

1. All reagents must be removed from refrigeration and allowed to come to room temperature (21 - 25 ºC) before use. Return all reagents to refrigerator promptly after use.
2. All samples and controls should be vortexed before use.
3. Dilute 25 mL of the 20X Wash Buffer to 0.5 L with distilled and/or deionized H20. Mix well.

ASSAY PROCEDURE

1. Place the desired number of strips into a microwell frame. Allow 4 Control determinations (one Negative Control, one Positive Control both in duplicate) per run. A reagent blank (RB) should be run on each assay. Check software and reader requirements for the correct Control configuration. Return unused strips to the sealable bag with desiccant, seal and immediately refrigerate.

Plate Configuration:

Plate Location	Sample Description	Plate Location	Sample Description
1A	RB	2A	Bovine #3
1B	NC	2B	Bovine #4
1C	NC	2C	Bovine #5
1D	RB	2D	Bovine #6
1E	PC	2E	Bovine #7
1F	PC	2F	Bovine #8
1G	Bovine #1	2G	Bovine #9
1H	Bovine #2	2H	Bovine #10

RB = Reagent Blank – well without serum addition run with all reagents. Utilized to blank reader.
NC = Negative Control, PC = Positive Control.

2. Dilute test sera, 1:50 (e.g., 4 µL + 196 µL) in Serum Diluent. Mix well. (For manual dilutions it is suggested to dispense the Serum Diluent into the test tube first and then add the bovine serum.)
3. To individual wells, add 100 µL of the appropriate Controls (PC and NC) and bovine sera. Add 100 µL of Serum Diluent to reagent blank well. Check software and reader requirements for the correct reagent blank well configuration.
4. Incubate each well at room temperature (21 to 25 ºC) for 120 minutes +/- 1 minute.
5. Aspirate or shake out liquid from all wells. If using semi-automated or automated washing equipment add 250-300 µL of diluted Wash Buffer to each well. Aspirate or shake out to remove all liquid. Repeat the wash procedure two times (for a total of three (3) washes) for manual or semi-automated equipment or four times (for a total of five (5) washes) for automated equipment. After the final wash, blot the plate on paper toweling to remove all liquid from the wells.

****IMPORTANT NOTE**: Regarding steps 5 and 8 - Insufficient or excessive washing will result in assay variation and will affect validity of results. Therefore, for best results the use of semi-automated or automated equipment set to deliver a volume to completely fill each well (250-300 µL) is recommended. A total of up to five (5) washes may be necessary with automated equipment. **Complete removal of the Wash Buffer after the last wash is critical for the accurate performance of the test. Also, visually ensure that no bubbles are remaining in the wells.**

6. Add 100 µL Conjugate to each well, including reagent blank well. Avoid bubbles upon addition as they may yield erroneous results.
7. Incubate each well at room temperature (21 to 25 ºC) for 30 minutes +/- 1 minute.
8. Repeat wash as described in Step 5.
9. Add 100 µL Chromogen/Substrate Solution (TMB) to each well, including the reagent blank well, maintaining a constant rate of addition across the plate.
10. Incubate each well at room temperature (21 to 25 ºC) for 1 – 15 minutes.
11. Stop reaction by addition of 50 µL of Stop Solution (1N H_2SO_4) following the same order of Chromogen/Substrate addition, including the reagent blank well. Tap the plate gently along the outsides, to mix contents of the wells. The plate may be held up to 1 hour after addition of the Stop Solution before reading.

RESULTS

Analysis

1. The Rats' Index Values are interpreted as follows:

OD Value	Results	Interpretation
<= Negative Control	Negative	No detectable antibody to Chlamydia Pneumoniae antigen by the ELISA test.
>= 1.3 folds of Negative OD value	Positive	Indicates presence of detectable antibody to Chlamydia Pneumoniae antigen by the ELISA test.

STORAGE

1. Store unopened kit between 2° and 8° C. The test kit may be used throughout the expiration date of the kit. Refer to the package label for the expiration date.
2. Unopened microassay plates must be stored between 2° and 8° C. Unused strips must be immediately resealed in a sealable bag with desiccant, and returned to storage at 2° and 8° C. If the bag is resealed with tape, the wells are stable for 30 days. If the bag is resealed with a heat sealer, the wells are stable until their labeled expiration date.
3. Store HRP Conjugate between 2° and 8° C.
4. Store the Positive and Negative Controls between 2° and 8° C.
5. Store Serum Diluent and 20X Wash Buffer between 2° and 8° C.
6. Store the Chromogen/Substrate Solution between 2° and 8° C. The reagent should remain closed when not in use. If allowed to evaporate, a precipitate may form in the reagent wells.
7. Store 1X (diluted) Wash Buffer at room temperature.