

# CANINE ALBUMIN

## Immunoperoxidase Assay for Determination of Albumin in Dog Samples

### DIRECTIONS FOR USE

Version3 L9.0 - 7C1Q1

For Research Use Only, NOT for Diagnostic Purposes

***Please Read this Package Insert Completely Before Using This Product***

### INTENDED USE

The Albumin test kits are a highly sensitive two-site enzyme linked immunoassay (ELISA) for measuring Albumin in biological fluid of Dogs.

### INTRODUCTION

Albumin is an amazing polyfunctional protein contributing to homeostasis through mechanisms of hemodynamics, transport and nutrition. Albumin is found both intra and extracellularly in all mammals and many lower vertebrates. It is a molecule of about 67,000 daltons, synthesized by the liver. Normally only very trace amounts of albumin escape reabsorption by kidney glomeruli and is excreted into the urine. Many occult diseases can cause kidney damage which may result in excessive amounts of serum proteins, including albumin, to be excreted by the kidney and into the urine. This ELISA kit can be used to measure albumin in serum, tissue extracts and other biological fluids.

### PRINCIPLE OF THE ASSAY

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



Figure 1.

### 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. ENZYME-ANTIBODY CONJUGATE 100X**  
One vial containing 150  $\mu$ L of affinity purified anti-Dog Albumin antibody conjugated with horseradish peroxidase in a stabilizing buffer.

#### 4. CHROMOGEN-SUBSTRATE SOLUTION

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

#### 5. STOP SOLUTION

One vial containing 12 ml 0.3 M sulfuric acid.

**WARNING: Avoid contact with skin.**

#### 6. ANTI-DOG ALBUMIN ELISA MICRO PLATE

Twelve removable eight (8) well micro well strips in well holder frame. Each well is coated with affinity purified anti-Dog Albumin.

#### 7. DOG ALBUMIN CALIBRATOR

One vial containing a lyophilized Dog Albumin calibrator.

**FOR IN VITRO USE ONLY**

### REAGENT PREPARATION

#### 1. DILUENT CONCENTRATE

The Diluent Solution supplied is a 5X Concentrate and must be diluted 1/5 with distilled or deionized water (1 part buffer concentrate, 4 parts dH<sub>2</sub>O).

#### 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 parts dH<sub>2</sub>O). 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. ENZYME-ANTIBODY CONJUGATE

Calculate the required amount of working conjugate solution for each microtitre plate test strip by adding 10 µL Enzyme-Antibody Conjugate to 990 µL of 1X Diluent for each test strip to be used for testing. Mix uniformly, but gently. Avoid foaming.

#### 4. CHROMOGEN-SUBSTRATE SOLUTION

Ready to use as supplied.

#### 5. STOP SOLUTION

Ready to use as supplied.

#### 6. ANTI-DOG ALBUMIN 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 along with desiccant.

#### 7. DOG ALBUMIN CALIBRATOR

Add 2.0 ml of distilled or de-ionized water to the Dog Albumin Calibrator and mix gently until dissolved. The calibrator is now at a concentration of 96.8 µg/ml **(the reconstituted calibrator should be aliquoted and frozen if future use is intended)**. Dog Albumin standards need to be prepared immediately prior to use (see the following chart). Mix well between each step. Avoid foaming.

Standard	ng/ml	Volume added to 1x Diluent	Volume of 1x Diluent
6	400	3 µl Dog Albumin Calibrator	723 µl
5	200	300 µl standard 6	300 µl
4	100	300 µl standard 5	300 µl
3	50	300 µl standard 4	300 µl
2	25	300 µl standard 3	300 µl
1	12.5	300 µl standard 2	300 µl
0	0		600 µl

### 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. ENZYME-ANTIBODY CONJUGATE

Undiluted horseradish peroxidase anti-Albumin conjugate should be stored at 4-8°C and **diluted immediately prior to use**. The working conjugate solution is stable for up to 1 hour if stored in the dark.

#### 4. CHROMOGEN-SUBSTRATE SOLUTION

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

#### 5. STOP SOLUTION

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

## 6. ANTI-DOG ALBUMIN ELISA MICRO PLATE

Anti-Dog Albumin coated wells are stable until the expiration date, and should be stored at 4-8°C in sealed foil pouch with desiccant pack.

## 7. DOG ALBUMIN CALIBRATOR

**The lyophilized Dog Albumin calibrator should be stored at 4°C or frozen until reconstituted. The reconstituted calibrator should be aliquoted out and stored frozen (Avoid multiple freeze-thaw cycles).** The working standard solutions should be prepared immediately prior to use and are stable for up to 8 hours.

## INDICATIONS OF INSTABILITY

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

## SPECIMEN COLLECTION AND HANDLING

Blood should be collected by venipuncture. The serum should be separated from the cells after clot formation by centrifugation. For plasma samples, blood should be collected into a container with an anticoagulant and then centrifuged. Care should be taken to minimize hemolysis, excessive hemolysis can impact your results. For urine samples, collect using standard techniques. The samples should be centrifuged to remove any possible debris. Assay immediately or aliquot and store samples at -20°C. Avoid repeated freeze-thaw cycles.

### 1. Precautions

For any sample that might contain pathogens, care must be taken to prevent contact with open wounds.

### 2. Additives and Preservatives

No additives or preservatives are necessary to maintain the integrity of the specimen. Avoid azide contamination.

### 3. Known interfering substances

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

## MATERIAL PROVIDED

See "REAGENTS"

## MATERIALS REQUIRED BUT NOT PROVIDED

- Precision pipette (2µL to 200µL) for making and dispensing dilutions
- Test tubes
- Microtitre washer/aspirator
- Distilled or Deionized H<sub>2</sub>O
- Microtitre Plate reader
- Assorted glassware for the preparation of reagents and buffer solutions
- Timer

## ASSAY PROTOCOL

### DILUTION OF SAMPLES

The assay for quantification of albumin in urine, serum or plasma requires that each test sample be diluted before use. A 1/1,000 dilution is appropriate for most urine samples while serum or plasma samples may need to be diluted 1/20,000. For absolute quantification, samples that yield results outside the range of the standard curve, a lesser or greater dilution might be required.

1. To prepare a 1/1,000 dilution of your sample, transfer 5 µL of sample to 495 µL of 1X diluent. This gives you a 1/100 dilution. Next, dilute the 1/100 sample by transferring 40 µL, to 360µL of 1X diluent. You now have a 1/1,000 dilution. Mix thoroughly at each stage.
2. To prepare a 1/200,000 dilution of your sample transfer 2 µL of sample to 1,998µL of 1X diluent. This gives you a 1/1,000 dilution. Next, dilute the 1/1,000 sample by transferring 2 µL, to 398µL of 1X diluent. You now have a 1/200,000 dilution. Mix thoroughly at each stage.

## PROCEDURE

1. **Bring all reagents to room temperature before use.**
2. Pipette 100 µL of
  - Standard 0 (0.0 ng/ml) in duplicate
  - Standard 1 (12.5 ng/ml) in duplicate
  - Standard 2 (25 ng/ml) in duplicate
  - Standard 3 (50 ng/ml) in duplicate
  - Standard 4 (100 ng/ml) in duplicate
  - Standard 5 (200 ng/ml) in duplicate
  - Standard 6 (400 ng/ml) in duplicate

3. Pipette 100  $\mu$ L of sample (in duplicate) into pre designated wells.

4. Incubate the micro titer plate at room temperature for sixty ( $60 \pm 2$ ) minutes. Keep plate covered and 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 then pour/shake out the contents in a waste container. Follow this by sharply striking the wells on absorbent paper to remove residual buffer. Repeat 3 times for a total of four washes.

7. Pipette 100  $\mu$ L of appropriately diluted Enzyme-Antibody Conjugate to each well. Incubate at room temperature for thirty ( $30 \pm 2$ ) minutes. Keep plate covered in the dark and level during incubation.

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

9. Pipette 100  $\mu$ L of TMB Substrate Solution into each well.

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

11. After ten minutes, add 100  $\mu$ L of Stop Solution to each well.

12. Determine the absorbance (450 nm) of the contents of each well. Calibrate the plate reader to manufacturer's specifications.

## 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.

## 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. Interpolate test sample values from standard curve. Correct for sera dilution factor to arrive at the Albumin concentration in original samples.

## 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, and accuracy of reagent and sample pipettings, washing technique, incubation time or temperature.

3. Do not mix or substitute reagents with those from other lots or sources.



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