

## Pana-test Canine Albumin

### 1. Introduction

Increases of urinary albumin are associated with the leakage of plasma proteins into urine due to lesions of the glomerular basement membranes. This phenomenon can act as an indicator for kidney injury, such as glomerular inflammation, renal amyloidosis and diabetic glomerular nephropathy. This ELISA kit bearing a specific antibody to canine albumin, can detect low levels of albumin, which never be detected by using testing papers for urinary proteins.

### 2. Characteristics

- This kit includes an exclusive reagent for quantitative determination of canine albumin.
- No specific facility is necessary.

### 3. Components of the Kit

- ELISA plate (anti-canine albumin antibody-coated microplate) ..... 1 plate
- Standard canine albumin (4 µg/mL) for 2 mL (lyophilized)..... 1 vial
- Sample diluent concentrate, 40 mL (5-fold concentrated, for 200 mL use)..... 1 vial
- Enzyme-labeled antibody (peroxidase-conjugated canine albumin )  
for 6 mL (lyophilized) ..... 1 vial
- Chromogen solution (containing 13.2 mg of 3,3',5,5'-tetramethylbenzidine in 0.5 mL of  
*N,N*-dimethylformamide) ..... 1 vial
- Substrate solution, 20 mL (containing 0.0083 w/v% hydrogen peroxide)..... 1 vial
- Washing buffer concentrate, 40 mL (10-fold concentrated PBS-Tween 20, for 400 mL use)..... 1 vial
- Stop solution, 15 mL (1 mol/L sulfuric acid)..... 1 vial

## 4. Reagent Preparation

| Component                    | Preparation   | Reagent prepared                          | Storage condition and stability                                 |
|------------------------------|---|---|---|
| ① ELISA plate                | Wait until the plate reaches to room temperature.<br>Add 300 $\mu$ L of wash buffer to each well just before use, and leave for 10 minutes.           | Anti-canine albumin antibody-coated plate | Prepare a required number of strip only immediately before use. |
| ② Standard canine albumin    | Add accurately 2.0 mL of purified water <sup>1)</sup> to the vial, and mix it thoroughly for complete dissolution.<br>Be careful not to form bubbles. | Standard canine albumin (4 $\mu$ g/mL)    | Stable in a refrigerator (2 to 10°C) for one week               |
| ③ Sample diluent concentrate | Add the entire volume of the concentrate (40 mL) into 160 mL of purified water, and mix it thoroughly.  | Sample diluent                            | Stable in a refrigerator (2 to 10°C) for one week               |
| ④ Enzyme-labeled antibody    | Add accurately 12 mL of purified water to the vial, and mix it thoroughly.  | Enzyme-labeled antibody solution          | Stable in refrigerator (2 to 10°C) for one week                 |
| ⑤ Chromogen solution         | Add 100 $\mu$ L of the chromogen solution into 10 mL of the substrate solution.   | Chromogenic substrate solution            | Freshly prepare, just before use.                               |
| ⑥ Substrate solution         |   |   |   |
| ⑦ Wash buffer concentrate    | Add the entire volume of the concentrate (40 mL) into 360 mL of purified water, and mix it thoroughly.  | Wash buffer <sup>2)</sup>                 | Stable at room temperature for one week                         |
| ⑧ Stop solution              | Use it as it is   |   | Stable at room temperature                                      |

1) Distilled or deionized water

2) PBS containing 0.05 v/v% Tween 20

NOTE: \*: Distilled or deionized water

All reagents should be allowed to equilibrate to room temperature before use.

Disused strips should be closed up in the foil pouch and stored at 2 to 10°C under dark.

Immediately use the chromogenic substrate solution after mixing ⑤ with ⑥.

## 5. Supplies Required

- Micropipettes and pipette tips (50  $\mu$ L, 100 to 1,000  $\mu$ L)
- Blowout pipettes (1 mL, 10 mL)
- Graduated cylinder (500 mL)
- Squir bottle, manifold dispenser, or automated microplate washer
- Multi-channel pipette
- Microplate reader capable of measurement at or near 450 nm
- Distilled or deionized water

## 6. Assay Procedure

### 6.1 Preparation of Standard Canine Albumin Solutions

Reconstitute the standard canine albumin with accurately 2.0 mL of deionized or distilled water, producing a 4 µg/mL standard. Swirl or mix gently and leave for a while to ensure complete reconstitution. Make serial dilutions of the 4 µg/ mL standard with the sample diluent, to prepare the standard solutions at 2000, 1000, 500, 250, 63 and 31 ng/mL.

Distribute the sample diluent to the 0 ng/mL standard.

### 6.2 Sample Dilution

#### Urine sample

Store the samples below -20°C.

ex.) Add 190 µL of the sample diluent to 10 µL of urine (20-fold dilution).

If you suspect a prolactin concentration in a test sample exceeds the highest point (4 µg /mL) of the standard curve, the test sample should be diluted with the sample diluent.

(As carry-over of the sample may be possible, it is recommended to replace the tip for each dilution.)

### 6.3 Assay Protocol

Bring all reagents and samples to room temperature before use. It is recommended that all samples, including the standards, are assayed in duplicate.

- 1) Add 300 µL of the wash buffer to each well of the ELISA plate. Incubate for 10 minutes at room temperature. (no adverse effect, even if left standing for up to 30 minutes.)
- 2) Aspirate each well to remove the solution.
- 3) Add 50 µL of the standard rat albumin or unknown samples to each well, and add 50 µL of the enzyme-labeled antibody solution to each well and mix, and incubate for 1 hours at room temperature.
- 4) Aspirate each well and wash the wells with wash buffer (300 µL/well). Repeat the washing procedure twice more. The complete removal of an aqueous fluid in each wash is essential for good performance.
- 5) Wash the wells as in step 4.
- 6) Add 100 µL of the chromogenic substrate solution to each well and incubate at room temperature for 15 minutes.
- 7) Add 50 µL of the stop solution to each well.
- 8) Measure an absorbance at 450 nm ( $A_{450}$ ) with a microplate reader.

## 7. Calculation of results

- 1) Average the duplicate reading for each standard and sample.
- 2) Plot the values of  $A_{450}$  (Y-axis) versus the concentrations of the standard solutions (X-axis), to draw a standard curve.
- 3) Apply an  $A_{450}$  value of each sample in the standard curve, so as to read a canine albumin

concentration in the sample.

- 4) Multiply the albumin concentration by the dilution factor to get a albumin concentration in the urine sample.

## 8. Safety Warnings and Precautions

- Strictly observe the storage condition for each reagent.
- All reagents should be brought to room temperature before use.
- Use reagents after confirming complete dissolution and uniformity.
- Take care to not inflict damage on any well when aspirating the solution in each well.
- When measuring many samples in one assay batch, the time period of each reaction for all samples should be uniformed at a fixed time as designated.
- Prepare the standard curve freshly for every measurement.
- Prepare the substrate solution with a clean vessel.
- White powder may sometimes be found in the wells of ELISA plate. This is due to the dried blocking solution, but will have no effect on measurement.
- Take care to handle the stop solution, very harmful.

## 9. Performance Characteristics

### 9.1 Quantitative Range

31 – 2,000 ng/mL of canine albumin

### 9.2 Intra - assay Precision

#### Standards

| Canine albumin (ng/mL) | (Replicate) | A <sub>450</sub> (mean) | C.V. (%) |
|------------------------|-------------|-------------------------|----------|
| 0                      | (N=8)       | 1.634                   | 0.6      |
| 31                     | (N=8)       | 1.594                   | 1.9      |
| 62                     | (N=8)       | 1.464                   | 1.0      |
| 125                    | (N=8)       | 1.261                   | 1.6      |
| 250                    | (N=8)       | 0.965                   | 1.8      |
| 500                    | (N=8)       | 0.668                   | 1.8      |
| 1000                   | (N=8)       | 0.449                   | 1.3      |
| 2000                   | (N=8)       | 0.278                   | 2.5      |

#### Samples

| Sample | (Replicate) | A <sub>450</sub> |        | Albumin conc. (ng/mL) |        |
|--------|-------------|------------------|--------|-----------------------|--------|
|        |             | mean             | CV (%) | mean                  | CV (%) |
| A      | (N=8)       | 0.786            | 1.8    | 378                   | 3.2    |
| B      | (N=8)       | 0.692            | 2.9    | 469                   | 4.7    |
| C      | (N=8)       | 0.555            | 1.4    | 665                   | 2.4    |

C.V. = coefficient of variation

Sample A, B, and C: 20-fold diluent of canine urine (beagle, male, 6 months of age)

### 9.3 Inter-assay Precision

#### Standards

| Canine albumin (ng/mL) | (Replicate) | A <sub>450</sub> (mean) | C.V. (%) |
|------------------------|-------------|-------------------------|----------|
| 0                      | (N=8)       | 1.540                   | 4.6      |
| 31                     | (N=8)       | 1.478                   | 6.1      |
| 62                     | (N=8)       | 1.375                   | 4.9      |
| 125                    | (N=8)       | 1.194                   | 4.4      |
| 250                    | (N=8)       | 0.931                   | 3.7      |
| 500                    | (N=8)       | 0.648                   | 2.9      |
| 1000                   | (N=8)       | 0.431                   | 3.7      |
| 2000                   | (N=8)       | 0.272                   | 2.6      |

#### Samples

| Sample | (Replicate) | A <sub>450</sub> |        | Albumin conc. (ng/mL) |        |
|--------|-------------|------------------|--------|-----------------------|--------|
|        |             | mean             | CV (%) | mean                  | CV (%) |
| A      | (N=8)       | 0.766            | 4.2    | 375                   | 6.7    |
| B      | (N=8)       | 0.673            | 3.4    | 470                   | 4.5    |
| C      | (N=8)       | 0.538            | 2.8    | 675                   | 3.3    |

C.V. = coefficient of variation

Sample A, B, and C: 20-fold dilution of canine urine (beagle, male, 6 months of age)

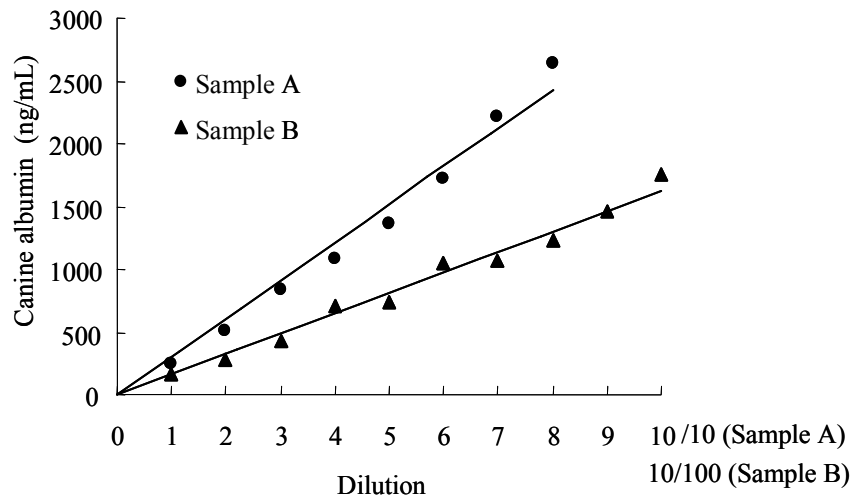
### 9.4 Recovery

Samples were prepared by spiking the five levels of standard canine albumin into canine urine (beagle, male, 6 months of age).

| Sample | Spiked amount (ng/mL) | Measured value (ng/mL) | Expected value (ng/mL) | Recovery (%) |
|--------|-----------------------|------------------------|------------------------|--------------|
| Urine  | 0                     | 184                    | -                      | -            |
|        | 63                    | 228                    | 247                    | 92.3         |
|        | 125                   | 294                    | 309                    | 95.2         |
|        | 250                   | 385                    | 434                    | 88.7         |
|        | 500                   | 617                    | 684                    | 90.2         |
|        | 1000                  | 1128                   | 1184                   | 95.3         |

## 9.5 Linearity of Dilution

Samples were prepared by a serial dilution of the canine urine samples with the sample diluent to 100-fold (beagle, male, 6 months of age).



## 10. Storage and Expiry

Store all reagents at 2-10°C under dark and use until a stated expiration date (one year after manufactured).

## 11. Package

96 tests per kit

<Distributed by>

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