BioLab Assays

Canine/Feline Clusterin ELISA

Cat. No.: BA2007

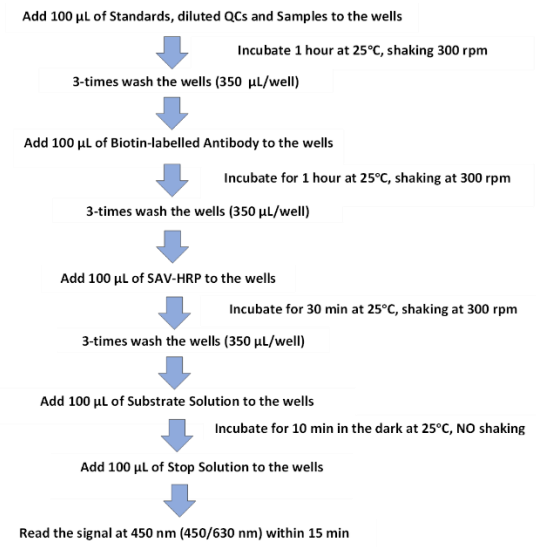
For research use only!

The BA2007 Canine/Feline Clusterin ELISA is a sandwich enzyme immunoassay for the quantitative measurement of canine/ feline clusterin in urine, serum and plasma.

Clusterin, also known as apolipoprotein J, is a widely expressed heterodimeric glycoprotein, important in tumorigenesis, apoptosis and immunoregulation. Clusterin is a cellular chaperon that stabilizes stressed proteins in a folding-competent state and protein has also been implicated in programmed cell death. Another defining prominent of clusterin is its induction in many severe physiological disturbance states including kidney degenerative diseases, prostate and vesicle carcinogenesis, ovarian cancer, and several neurodegenerative conditions (Alzheimer's disease) [1-3].
Recent findings demonstrate that high serum clusterin levels are connected to oxidation stress, vascular damage, sepsis and related mortality or significantly lower serum clusterin was observed in dogs with multicentric lymphoma (MLSA) [4,5].
Urinary clusterin has been approved as a biomarker to monitor drug-induced proximal tubular injury in rats by the U.S. Food and Drug Administration and the European Medicines Agency. Furthermore, urinary clusterin may also help to differentiate between tubular and glomerular forms of proteinuria [5-7].

PRINCIPLE OF CANINE/FELINE CLUSTERIN ELISA

In the Canine/Feline Clusterin ELISA, standards, quality controls and samples are incubated in microplate wells pre-coated with specific anti-canine/feline clusterin antibody. After 60 minutes incubation and washing, biotin labelled anti-canine/feline clusterin antibody is added and incubated with captured clusterin for 60 minutes. After another washing, streptavidin-HRP conjugate is added. After 30 minutes incubation and the last washing step, the remaining conjugate is allowed to react with the substrate solution (TMB). The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow product is measured. The absorbance is proportional to the concentration of clusterin. A standard curve is constructed by plotting absorbance values against concentrations of standards, and concentrations of unknown samples are determined using this standard curve.



Kit Contents

Item	***Qty.***
Antibody Coated Microtiter Plate	96 wells
Streptavidin-HRP Conjugate	13 mL
Biotin Labelled Antibody 50x conc.	1 vial
Biotin-Ab Diluent	13 mL
Master Standard (lyophilized)	1 vial
Quality Control A	1 vial
Quality Control B	1 vial
Dilution Buffer 10x conc.	13 mL
Wash Buffer 15x conc.	50 mL
Substrate Solution	13 mL
STOP Solution	13 mL

MATERIAL REQUIRED BUT NOT SUPPLIED

1. Glassware and test tubes
2. Microtiter plate washer
3. Precision pipettes (various volumes) with tips
4. Orbital shaker
5. Microtiter plate reader capable of measuring absorbance at 450 nm or 450/630 nm with software for data generation

WARNINGS AND PRECAUTIONS

1. For research use only
2. For professional laboratory use
3. The reagents with different lot numbers should not be mixed
4. To prevent cross sample contamination, use disposable labware and pipette tips
5. To protect laboratory stuff, wear protective gloves and protective clothing
6. The substrate solution should remain colourless, keep it protected from light
7. The test should be performed at standard laboratory conditions (temperature 25°C ±2°C).

STORAGE CONDITIONS

1. The kit must be stored at 2 – 8°C.
2. The opened components can be stored for one week at 2 – 8°C.

PREPARATION OF REAGENTS

- Use new pipette tip for pipetting different reagents and samples to prevent cross-contamination.
- All reagents and samples should be allowed to reach the temperature 25°C ±2°C.

Canine/Feline Clusterin Master Standard

Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution of standard!!! Reconstitute the lyophilized Master Standard with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam). The resulting concentration of the clusterin in the stock solution is 40 ng/ml.
Prepare set of standards using Dilution Buffer as follows:

	Volume of Standard	Dilution Buffer	Concentration
Std1	Standard 40 ng/mL (lyophilized)	See CofA	40 ng/ml
Std2	300 µL of Std1	300 µL	20 ng/ml
Std3	300 µL of Std2	450 µL	8 ng/ml
Std4	300 µL of Std3	300 µL	4 ng/ml
Std5	300 µL of Std4	300 µL	2 ng/ml
Std6	300 µL of Std5	300 µL	1 ng/ml
Blank	-	200 µL	0 ng/mL

Prepared Standards are ready to use, do not dilute them.

Quality Controls A and B

Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution and for current Quality Control concentration!!!
Reconstitute each Quality Control (A and B) with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam).
Reconstituted Quality Controls are ready to use, do not dilute them.

Note:
Concentration of analyte in Quality Controls need not be anyhow associated with normal and/or pathological concentrations in serum or another body fluid. Quality Controls serve just for control that the kit works in accordance with IFU and CoA and that ELISA test was carried out properly.

Biotin Labelled Antibody 50x Conc.

Prepare the working Biotin Labelled Antibody solution by adding 1 part Biotin Labelled Antibody Concentrate (50x) with 49 parts Biotin-Ab Diluent. Example: 260 µl of Biotin Labelled Antibody Concentrate (50x) + 13ml of Biotin-Ab Diluent for 12 strips (96 wells).

Preparation of Wash Buffer 1x

Prepare a working solution of Wash Buffer by adding 50 mL of Wash Buffer 15x conc. to 700 mL of deionized/ distilled water (dH2O). Mix well. Store at 4°C for two weeks or at -20°C for long term storage.

Preparation of Dilution Buffer 1x

Prepare a working solution of Dilution Buffer by adding 13 mL of Dilution Buffer 10x conc. to 117 mL of deionized/ distilled water (dH2O). Mix well. Store at 4 °C for two weeks or at -20 °C for long term storage.

PREPARATION OF SAMPLES

Urine samples
Dilute urine samples just prior to perform the test 30x with Dilution Buffer, e.g. 5 µl of sample + 145 µl of Dilution Buffer for singlets or 10 µl of sample + 290 µl of Dilution Buffer for duplicates. Mix well (not to foam). Vortex is recommended.

Stability and storage:
Urine samples should be assayed immediately after collection or stored at -70°C. Avoid repeated freeze/ thaw cycles.

Serum or plasma samples

Dilute serum or plasma samples just prior to perform the test 2500x with Dilution Buffer in two steps as follows:

Dilution A (50x):
Add 5 µl of sample into 245 µl of Dilution Buffer and mix well (not to foam). Vortex is recommended.
Dilution B (50x):
Add 5 µl of Dilution A into 245 µl of Dilution Buffer to prepare final dilution 2500x. Mix well (not to foam). Vortex is recommended.

Serum or plasma samples should be assayed immediately after collection or should be stored at -20°C, or preferably at -70°C for long-term storage. Avoid repeated freeze/thaw cycles, which may cause erroneous results. Avoid using hemolyzed or lipemic samples.

ASSAY PROCEDURE

1. Prepare the reagents as described in the previous chapter.
2. Pipette 100 µL of set of Standards, Quality Controls, diluted Samples and Dilution Buffer = Blank into each well. Incubate for **1 hour** at 25°C ±2°C, shaking at 300 rpm.
3. Wash the wells 3-times with 1x Wash Buffer (350 µL/well). When finished, tap the plate against the paper towel to remove the liquid completely.
4. Pipette 100 µL of Biotin Labelled Antibody into each well. Incubate for **1 hour** at 25°C ±2°C, shaking at 300 rpm.
5. Wash the wells as described in point 3.
6. Pipette 100 µL of HRP-labelled Antibody Conjugate into each well. Incubate for **30 minutes** at 25°C ±2°C, shaking at 300 rpm.
7. Wash the wells as described in point 3.
8. Pipette 100 µL Substrate solution, incubate for **10 min,** at 25°C ±2°C**.** Avoid exposure to the light during this step.
9. Pipette 100 µL of STOP solution.

Read the signal at 450 or 450/630 nm within 15 min.

	strip 1+2	strip 3+4	strip 5+6	strip 7+8	strip 9+10	strip 11+12
A	**Standard 40**	**Blank**	Sample 8	Sample 16	Sample 24	Sample 32
B	**Standard 20**	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
C	**Standard 8**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
D	**Standard 4**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
E	**Standard 2**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
F	**Standard 1**	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
G	**QC A**	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38
H	**QC B**	Sample 7	Sample 15	Sample 23	Sample 31	Sample 39

Figure 1: Example of a work sheet.

Typical standard curve

The standard curve needs to be measured in every test. Most of the microplate reader can automatically calculate the analyte concentration using 4-parameter algorithm or alternative functions to fit the standard points properly. The concentrations need to be multiplied by the dilution factor, either automatically by reader or manually.

The measured concentration of samples calculated from the standard curve must be multiplied by their respective dilution factor, because samples have been diluted prior to the assay, e.g. 7,1 ng/ml (from standard curve) x 10 (dilution factor) = 71 ng/ml.

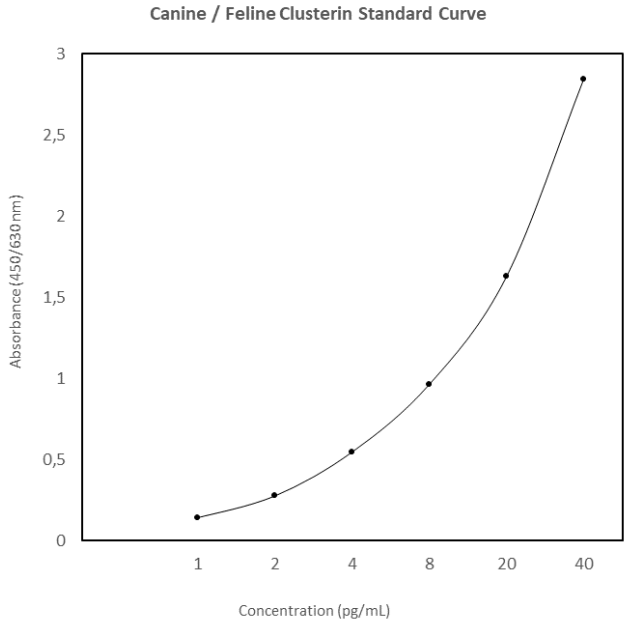


Figure 2: Typical Standard Curve for Canine/ Feline Clusterin ELISA.

PERFORMANCE CHARACTERISTICS

Typical analytical data of Canine/ Feline Clusterin ELISA are presented in this chapter.

Sensitivity

Limit of Detection (LOD) (defined as concentration of analyte giving absorbance higher than mean absorbance of blank* plus three standard deviations of the absorbance of blank: Ablank + 3xSDblank) is calculated from the real canine/feline clusterin values in wells and is 0.2 ng/ml.

Specificity

The antibodies used in this ELISA are specific for canine/ feline clusterin.

Precision

Intra-assay (Within-Run) (n=8)

Sample	Mean	SD	CV (%)
Urine 1	45 ng/ml	2.3	5.1
Serum 1	64.2 µg/ml	2.8	4.6

Inter-assay (Run-to-Run) (n=8)

Sample	Mean	SD	CV (%)
Urine 1	41 ng/ml	3.1	7.5
Serum 1	62.5 µg/ml	3.2	5.1

Spiking Recovery

Urine and serum samples were spiked with different amounts of clusterin and assayed.

Sample	Observed	Expected	Recovery O/E (%)
Urine 2	55.6 (ng/ml)	-	-
	129.2	135.6	95.3
	87.3	95.6	91.4
	70.6	75.6	93.5
Serum 1	63.1 (µg/ml)	-	-
	130.2	146.1	91.0
	94.4	103.1	91.5
	79.5	83.1	95.6

Linearity

Serum samples were serially diluted with Dilution Buffer and assayed.

Sample	Dilution	Observed	Expected	Recovery O/E (%)
Urine 3	-	64.6 (ng/ml)	-	-
	2x	30.1	32.3	93.2
	4x	14.9	16.2	91.2
	8x	7.0	8.1	86.4
Serum 1	-	58.2 (µg/ml)	-	-
	2x	30.2	29.1	103.8
	4x	16.1	14.5	111.0
	8x	7.4	7.3	101.4

Reference range

It is recommended that each laboratory include its own panel of control samples in the assay. Each laboratory should establish its own normal and pathological reference ranges for canine / feline clusterin levels with the assay.

DEFINITION OF THE STANDARD

Standard in this assay is canine / feline serum based native clusterin.

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