Canine Amyloid-Beta Aβ(1–40) ELISA Protocol

FIVEphoton Biochemicals

For research use only.
Not for diagnostics.

Part No. cAB1-40ELISA

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Store: 4°C
Safety: Stop solution contains acid. Avoid all contact and inhalation.

Canine Amyloid-Beta 1- 40 (Aβ1-40) ELISA Kit

For research only. Not for diagnostic applications.

Storage: 4°C, six months after manufacture

Safety: Stop solution contains acid. Avoid eye and skin contact

Standard peptide concentration: 3.6ng/ml

Assay range: 50pg/ml to 4ng/ml. 96T

This ELISA kit is based on the double-antibody sandwich technique to detect canine amyloid-Beta 1-40 (Aβ1-40). The kit detects soluble Aβ1-40 in a variety of sources, including biological fluids, tissue homogenates and cell lysates. It can be used to measure canine Aβ1-40 expressed in transgenic mouse models as well as in recombinant cell culture systems. Additionally, Triton-based cell lysis buffers can be employed to extract Aβ1-40 and the resulting samples can be assayed by the kit.

Experimental Principles

Samples are applied to microelisa wells pre-coated with an affinity purified anti- canine amyloid-Beta 1-40(Aβ1-40) antibody. Samples are incubated, and after the incubation period, the wells are washed. A second anti-canine amyloid-Beta 1-40(Aβ1-40) antibody conjugated with HRP is added, followed by incubation and wash. Chromogen solutions A and B are added resulting in a color change to blue. A stop solution is applied to terminate the reaction, turning the solution to yellow. Absorbance readings at 450 nm are used to directly correlate the concentration of amyloid-Beta 1-40 in the samples to the standard peptide.

Materials supplied

1	Standard peptide（3.6ng/ml）	0.5ml	7	Chromogen Solution A	6ml
2	Standard diluent	1.5ml	8	Chromogen Solution B	6ml
3	Microelisa Strip plate	12well×8strips	9	Stop Solution	6ml
4	HRP-Conjugate Antibody	6ml	10	Instruction Manual	1
5	30×wash solution	20ml	11	Closure plate membrane	2
6	Sample diluent	6ml	12	Sealed bags	1

Materials required but not supplied

1. 37°C incubator
2. Standard microplate reader
3. Precision pipettes and disposable pipette tips
4. De-ionized water
5. Disposable tubes for sample dilution
6. Absorbent paper

Important notes and preparation for the assay

1. The kit should be equilibrated to room temperate for 30 min prior to performing the assay. Store opened microelisa plates in a sealed plastic bag at 4°C. A multi-channel sampler that has been calibrated for accuracy is a preferred method to apply samples. Plates should be sealed during the assay; wells should not be allowed to dry.
2. Perform dilution of the standards in separate tubes or 96-well plates, not in the ELISA wells. Transfer solutions simultaneously to the ELISA dish.
3. It is recommended that the experimenter perform preliminary tests to identify the sample dilution required to meet the assay range. Perform a preliminary assay with samples, using standards at the high and low dilution. Suspend and dilute experimental samples into the "sample diluent" provided with this kit to meet the requirements of the assay range. A dilution series of several samples may be required to identify the correct concentration that meets the assay range. Concentrate or dilute test samples if adjustment is needed. Set aside sufficient experimental sample for reserve to repeat the assay.
4. Determine whether the sample vehicle reacts with the assay: If needed, dilute the sample in sample diluent or prepare samples in another vehicle to prevent inadvertent experimental readings.
5. It is recommended that samples are assayed in duplicate to mitigate pipetting error.
6. Use new applicator tips and ELISA plate sealants to avoid cross-contamination.
7. Do not mix reagents from other ELISA kits.
8. Note that sodium azide in samples that is not washed away may inhibit HRP.
9. When calculating concentration of your sample, make sure to take into account the dilution factor.
10. If the wash solution crystallizes during storage at 4°C, heat the solution at 37°C and shake until crystals suspend.

Sample Preparation: Use 50 ml tubes and a desktop cell culture centrifuge for the following procedures.

1. **Serum**: Coagulate at room temperature for 10-20 min. Centrifuge for 20 min at 2000-3000 rpm. Collect supernatant to assay. If precipitation appears, centrifuge again. Assay the supernatant fraction.
2. **Plasma:** Use suitable EDTA or heparin as an anticoagulant. Mix for 10-20 min using a stir bar. Centrifuge for 20 min at 2000-3000 rpm. Collect supernatant to assay. If precipitation appears, centrifuge again.
3. **Urine**: Collect in a sterile container. Centrifuge 20-min at 2000-3000 rpm. Collect supernatant to assay. If precipitation appears, centrifuge again. Collect supernantant.
4. **Cell culture supernatant:**. Detection of secretory components: Centrifuge culture media for 20 min at 2000-3000 rpm. Collect supernatant to assay.
5. **Cell cytoplasm:** Dilute cell suspension with PBS (pH7.2-7.4) to a cell concentration of 1 million cells / ml. Perform repeated freeze-thaw cycles to fracture the cell membrane and to release intracellular components. Centrifuge for 20min at 2000-3000 rpm. Collect supernatant to assay. If precipitation appears, centrifuge again.
5. **Tissue:** Cut and weigh tissue slice. Add slice in PBS (pH7.2-7.4). Freeze rapidly with liquid nitrogen. Thaw sample to 2-8°C, add PBS and homogenize. Centrifuge for 20 min at 2000-3000rpm. Collect supernatant to assay.
6. Samples can be store at -80°C. Avoid repeated freeze-thaw cycles. You may aliquot samples for later ELISA assays.
7. **Avoid denaturing cell lysis buffers that contain SDS such as RIPA buffer.**

Sample Application to ELISA dish

Standard and Sample Preparation. Prepare the blanks, standards and samples as described below in a separate 96-well dish prior to simultaneous transfer to the ELISA dish.

Assay procedure

1. Set aside and mark 14 wells for standard peptide dilutions. Configure seven concentrations of standard peptide in duplicate, suspended and mixed as indicated below in separate tubes, or in separate wells of a 96-well dish. Do not use the ELISA wells directly to perform the dilutions: The final total volume in each well should be 50μl.

Table 2. Standard dilutions

3.6ng/ml	Standard No. 7	50μl standard Peptide
2.4g/ml	Standard No. 6	100μl standard peptide + 50μl standard diluent, mix. Remove 100μl to make Standard No. 5.
1.6ng/ml	Standard No. 5	100μl Standard No. 6 + 50μl standard diluent, mix. Remove 100μl to make Standard No. 4.
800pg/ml	Standard No. 4	100μl Standard No. 5 + 100 μl Standard diluent, mix. Remove 50μl to make Standard No. 3. Remove and discard 100μl.
400pg/ml	Standard No. 3	50μl Standard No. 4 + 50μl Standard diluent, mix. Remove 50μl to make Standard No. 2.
200pg/ml	Standard No. 2	50μl Standard No. 3 + 50μl Standard diluent, mix. Remove 50μl to make standard 1.
100pg/ml	Standard No. 1	50μl of Standard No. 2 + 50μl Standard diluents, mix. Discard 50μl.

2. Set up 2 blank wells separately. In blank wells, add 40μl sample diluent and 10μl solution with the same ratio of sample vehicle to sample diluent used in the experimental samples; do not add sample. Perform all other procedures of the assay in the “blank wells,” except do not add HRP conjugated antibody to the blank wells.

3. For wells that will hold experimental samples, prepare 10μl of sample diluted in 40 μl sample diluent. This creates a 5X dilution factor which should be accounted for later when calculating sample concentration.

4. Transfer the blank well, standard solutions, and experimental samples simultaneously to the ELISA dish.

5. Use the closure membrane to enclose the plate, mix gently with a rotating table, and incubate for 30 min at 37°C, or 1 hr at RT.

6. Dilute the 30X Wash Solution with dH_20. Make 3 ml of diluted wash solution for each assay well.

7. After the first incubation period, discard the liquid in the wells by gently aspirating. Then turn the plate upside down and gently pat dry the plate with an absorbent paper. To wash the wells, fill each well with 100 μl of diluted Wash Solution, oscillate gently with the rocker table for 30 sec, and then aspirate off the liquid. Pat dry the microplate with absorbent paper. Repeat the wash steps 5 times.

8. Simultaneously transfer 50 μl HRP-conjugate reagent to each well, except for the blank wells. Rotate gently for 30 min at 37°C or 1 hr at RT.

9. Wash the wells as described in step 7. As in step 7, wash the wells five times. Remove liquid from the wells.

10. Simultaneously transfer 50μl of Chromogen Solution A and 50μl of Chromogen Solution B to each well (prepare these solutions beforehand in a separate 96-well dish). Gently mix for 15 min at 37°C in the dark.

11. Simultaneously add 50μl of Stop Solution to each well. Upon addition of stop solution, the blue color should immediately change to yellow.

12. Measure the optimal density (OD) at 450 nm within 15 minutes of adding the stop solution. Set the blank wells as zero.

13. If sample readings are higher than the most concentrated standard, you may wish to dilute the samples and assay again.

Data Analysis

1. Compile a standard curve using the blank standard solutions and the corresponding OD values. You may wish to calculate a linear regression equation from the standard curve to determine the concentration of your samples. Remember that samples were diluted 5 fold in the assay in your final calculation. Other forms of analysis to calculate concentrations of your samples can also be applied.

Flow chart of the procedures

Prepare standards, blank and samples

Add samples to wells, incubate for 1 hr at RT or 30 min at 37°C.

Wash each well five times.

Add HRP conjugate antibody to each well, incubate for 1 hr at RT or 30 min at 37°C.

Wash each well five times

Add chromogen solutions A and B, 15 min at 37°C

Add stop solution

Measure OD 450 nm within 15 min