Dog IL1a (Interleukin 1 Alpha) Elisa Kit
EK245361

Product Information

- Assay Type: Sandwich
- Species: Dog
- Standard: 500
- Sensitivity: 3.3
- Detection Range: 7.82-500 pg/mL
- Sample Type: Serum, Plasma, Tissue Homogenates, Cell Lysates, Cell Culture Supernates, other Biological Fluids
- Size: 96 Wells

Components

Assay plate (12 × 8 coated Microwells)	1
Standard (Lyophilized)	2 vials
Biotinylated Antibody (100×)	120 µL
Streptavidin-HRP (100×)	120 µL
Standard/Sample Diluent Buffer	20 ml
Biotinylated Antibody Diluent	12 ml
HRP Diluent	12 ml
Wash Buffer (25×)	20 ml
TMB Substrate Solution	10 ml
Stop Reagent	6 ml
Plate Covers	2

Test Principle

This assay uses the Sandwich enzyme immunoassay method. The provided microtiter plate in the kit is pre-coated with IL1a (Interleukin 1 Alpha). Standards or samples are added to the appropriate wells of the microtiter plate, along with a biotin-conjugated antibody specific to IL1a (Interleukin 1 Alpha). Then, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each well and incubated. Following this, TMB substrate solution is added. Only the wells that contain IL1a (Interleukin 1 Alpha), biotin-conjugated antibody, and enzyme-conjugated Avidin will show a color change. The enzyme-substrate reaction is stopped by adding a sulfuric acid solution, and the color change is measured spectrophotometrically at a wavelength of 450nm ± 10nm. The concentration of IL1a (Interleukin 1 Alpha) in the samples is determined by comparing the optical density (OD) of the samples to the standard curve.

Sample Collection

Serum - Use a serum separator tube and allow samples to clot for two hours at room temperature or overnight at 4oC before centrifugation for 20 minutes at approximately 1,000×g. Assay freshly prepared serum immediately or store samples in aliquot at -20oC or -80oC for later use. Avoid repeated freeze/thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1,000×g at 2-8oC within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquot at -20oC or -80oC for later use. Avoid repeated freeze/thaw cycles.

Tissue homogenates - The preparation of tissue homogenates will vary depending upon tissue type.

1. Tissues were rinsed in ice-cold PBS to remove excess blood thoroughly and weighed before homogenization.
2. Minced the tissues to small pieces and homogenized them in fresh lysis buffer (Different lysis buffer needs to be chosen based on subcellular location of the target protein) (w:v = 1:20-1:50, e.g. 1mL lysis buffer is added in 20-50mg tissue sample) with a glass homogenizer on ice (Micro Tissue Grinders works, too).
3. The resulting suspension was sonicated with an ultrasonic cell disrupter till the solution is clarified.
4. Then, the homogenates were centrifuged for 5 minutes at 10,000×g. Collect the supernates and assay immediately or aliquot and store at ≤-20oC.

Cell Lysates - Cells need to be lysed before assaying according to the following directions.

1. Adherent cells should be washed by cold PBS gently, and then detached with trypsin, and collected by centrifugation at 1,000×g for 5 minutes (suspension cells can be collected by centrifugation directly).
2. Wash cells three times in cold PBS.
3. Resuspend cells in fresh lysis buffer with concentration of 10^7 cells/mL. If it is necessary, the cells could be subjected to ultrasonication till the solution is clarified.
4. Centrifuge at 1,500×g for 10 minutes at 2-8oC to remove cellular debris. Assay immediately or aliquot and store at ≤-20oC.

Urine - Collect the first urine of the day (mid-stream) and discharge it directly into a sterile container. Centrifuge to remove particulate matter, assay immediately or aliquot and store at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Saliva - Collect saliva using a collection device or equivalent. Centrifuge samples at 1000 × g at 2-8°C for 15 minutes. Remove particulates and assay immediately or store samples in aliquot at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Feces - Dry feces were collected as much as possible, weighing more than 50 mg. The feces were washed three times with PBS (w:v = 1:9, e.g. 900 μL lysis buffer is added in 100 mg feces), sonicated(or mashed)and centrifuged at 5000×g for 10 minutes, where the supernatant was collected for testing

Cell culture supernates and other biological fluids - Centrifuge samples for 20 minutes at 1,000×g. Collect the supernates and assay immediately or store samples in aliquot at -20oC or -80oC for later use. Avoid repeated freeze/thaw cycles.

Note

1. Samples to be used within 5 days may be stored at 4oC, otherwise samples must be stored at -20oC (≤1 month) or -80oC (≤2 months) to avoid loss of bioactivity and contamination.
2. Sample hemolysis will influence the result, so hemolytic specimen should not be used.
3. When performing the assay, bring samples to room temperature.
4. It is highly recommended to use serum instead of plasma for the detection based on quantity of our in-house data.

Reagent Preparation

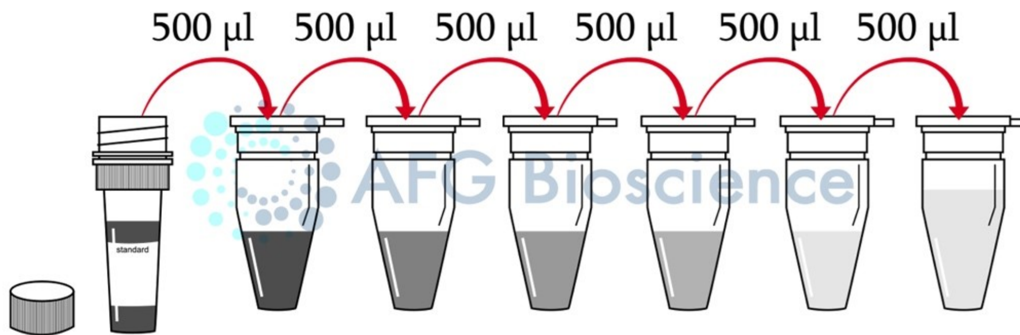
- Ensure all kit components and samples are brought to room temperature (18-25°C) before use. Thoroughly dissolve and mix all components before utilizing the kit.
- If the entire kit will not be used at once, only remove the necessary strips and reagents for the current experiment. Store the remaining strips and reagents as directed.
- Dilute the 25× Wash Buffer into 1× Wash Buffer with double distilled water.

Standard Working Solution

First, centrifuge the Standard at 1000 x g for 1 minute. Then, reconstitute the Standard by adding 1.0 ml of Standard Diluent Buffer. Allow the mixture to sit for approximately 10 minutes at room temperature, and gently shake it (avoiding foaming).

Prepare 7 tubes with 0.5 ml of Standard Diluent Buffer in each and create a series of dilutions using the Diluted Standard. Remember to thoroughly mix each tube before transferring the solution to the next tube by pipetting the solution up and down several times. Set up 7 points of Diluted Standard, decreasing the concentration each time, and use a Blank with no standard for comparison. It's important to use a new Standard Solution for each experiment to ensure accurate results. When diluting the Standard from high concentration to low concentration, replace the

pipette tip for each dilution. Additionally, make sure not to transfer solution into the Blank tube from the previous one.



pg/ml	500	250	125	62.5	31.25	15.625	7.8125

- Before using the stock Biotinylated Antibody and Streptavidin-HRP, give them a quick spin. Dilute them to the working concentration 100-fold with Biotinylated Antibody Diluent and HRP Diluent.
- When using the TMB Substrate Solution, be sure to aspirate the required amount with sterilized tips and avoid returning any leftover solution to the vial.

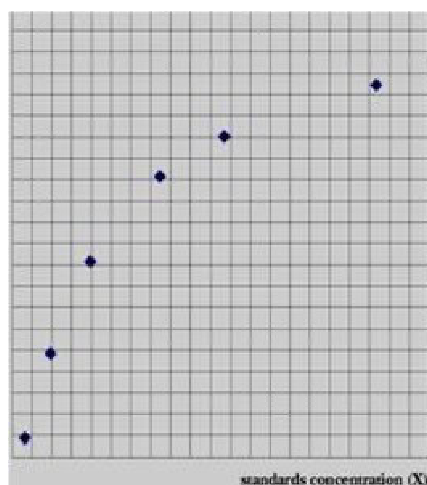
Assay Procedure

1. Prepare the micro ELISA plate by allocating wells for the Diluted Standard, Blank, and Sample. Allocate 7 wells for the Standard and 1 well for the Blank.
2. Add 100 µL of Standard Working Solution or 100 µL sample to each well. Cover with the Plate Cover, and incubate for 80 minutes at 37°C. When adding solutions, be careful to avoid touching the inside wall and causing foaming.
3. Pour out the liquid from each well, then wash by adding 200 µL of 1x Wash Solution to each well and letting it sit for 1-2 minutes. Remove the remaining liquid from all wells by placing the plate onto absorbent paper. Repeat this process 3 times to ensure complete removal of liquid at each step. After the last wash, invert the plate and blot it against clean paper towels to remove excess liquid.
4. Add 100 µL of Biotinylated Antibody Working Solution (1x) to each well, cover with the Plate Cover, and incubate at 37°C for 50 minutes.
5. Repeat the aspiration and wash process a total of 3 times as conducted in step 3.
6. Add 100 µL of Streptavidin-HRP Antibody Working Solution to each well, cover with the Plate Cover, and incubate at 37°C for 50 minutes.
7. Repeat the aspiration and wash process a total of 5 times as conducted in step 3.

8. Add 90 µL of TMB Substrate Solution to each well, cover with a new Plate Cover, and incubate for 20 minutes at 37°C (do not exceed 30 minutes) in the dark. The liquid will turn blue by the addition of TMB Substrate Solution. Preheat the Microplate Reader for about 15 minutes before OD measurement and avoid placing the plate in direct light.
9. Add 50 µL of Stop Reagent to each well. The liquid will turn yellow by the addition of Stop Reagent. Mix the liquid by tapping the side of the plate. If the color change doesn't look even, simply tap the plate gently to make sure the mixing is thorough. The insertion order of the Stop Reagent should be the same as that of the TMB Substrate Solution.
10. Run the microplate reader and conduct measurements at 450 nm immediately.

Calculation of Results

Take the standard concentration as the horizontal, the OD value for the vertical, draw the standard curve on graph paper, find out the corresponding concentration according to the sample OD value by the sample curve, multiplied by the dilution multiple, or calculate the straight line regression equation of the standard curve with the standard concentration and the OD value, with the sample OD value in the equation, calculate the sample concentration, multiplied by the dilution factor, the result is the sample actual concentration.



Precision

Intra-assay Precision (Precision within an assay): CV%<8%

Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays):CV%<10%

Three samples of known concentration were tested in forty separate assays to assess inter-assay precision.

Storage

Store the kit at 2-8°C immediately upon receipt. For long-term storage the entire kit can be stored at -20°C within its shelf life.

Download our ELISA technical hints, troubleshooting tips
www.afgsci.com/troubleshooting/

For technical support contact information, visit: https://www.afgsci.com/contact/