PROMPT
Proteins for Research

ISO 9001:2015 ISO 13485:2016

Dog SP (Substance P) ELISA Kit

For research use only. Not intended for diagnostic use.

Cat: OPK6297

Detection Range: 78.13 – 5000pg/mL.

Sensitivity: 46.88 pg/mL.

Repeatability: Coefficient of variation is 10%

Kit Components & Storage

An unopened kit can be stored at 2-8℃ for 1 year. If the opened kit is not used up, store the items separately according to the following conditions.

Reagents	Quantity		Storage Condition
	96T	48T	
Micro ELISA Plate	12 strips x 8 wells	6 strips x 8 wells	4℃ (6 months) -20℃ (12 months)
Reference Standard	2vials	1vial	4℃ (6 months) -20℃ (12 months)
Concentrated Biotinylated Detection Ab (100×)	60µL	60µL	4℃ (6 months) -20℃ (12 months)
Concentrated HRP Conjugate (100×)	120µL	60µL	4℃ (6 months) -20℃ (12 months)
Reference Standard & Sample Diluent	20mL	20mL	4℃ (12 months)
Biotinylated Detection Ab Diluent	13mL	13mL	4℃ (12 months)
HRP Conjugate Diluent	13mL	13mL	4℃ (12 months)
Concentrated Wash Buffer (25×)	30mL	30mL	4℃ (12 months)
Substrate Reagent	10mL	10mL	4℃ (12 months, store in Dark)
Stop Solution	10mL	10mL	4℃ (12 months)
Plate Sealer	5pieces	5pieces	RT

Materials Required, Not Supplied

1. Microplate reader capable of measuring absorbance at 450 ± 10 nm.
2. High-speed centrifuge.
3. Electro-heating standing-temperature cultivator.
4. Absorbent paper.
5. Double distilled water or deionized water.
6. Single or multi-channel pipettes with high precision and disposable tips.
7. Precision pipettes to deliver 2µL to 1mL volumes.

Safety Notes

1. This kit is only used for lab research and development and should not be used for human or animals.
2. Reagents should be regarded as hazardous substances and should be handled carefully and correctly.
3. Gloves, lab coats, and goggles should always be worn to avoid skin and eyes coming into contact with Reagents. In case of contact, wash thoroughly with water.

Test Principle

This ELISA kit uses the Competitive-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with Dog SP. During the reaction, Dog SP in the sample or standard competes with a fixed amount of Dog SP on the solid phase supporter for sites on the Biotinylated Detection Ab specific to Dog SP. Excess conjugate and unbound sample or standard are washed away, and Avidin-Horseradish Peroxidase (HRP) conjugate are added to each micro plate well and incubated. Then a TMB substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of stop solution and the colour turns from blue to yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm. The concentration of Dog SP in tested samples can be calculated by comparing the OD of the samples to the standard curve.

Sample Collection and Storage

Serum - Samples should be collected into a serum separator tube. After clotting for 2 hours at room temperature or overnight at 4℃, and then centrifuging at 1000 × g for 20 minutes. Assay freshly prepared serum immediately or store samples in aliquot at -20℃ or -80℃ for later use. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples at 1000 × g and 2- 8℃ for 15 minutes within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquot at -20℃ or -80℃ for later use. Avoid repeated freeze-thaw cycles.

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

1. Rinse the tissues in pre-cooled PBS to completely remove excess blood, and weigh them before homogenization.
2. Mince 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) (PBS can be used as the lysis buffer of most tissues) (w:v = 1:9, e.g. 900µL lysis buffer is added in 100mg tissue sample) with a glass homogenizer on ice (micro tissue grinders, too).
3. Ultrasound the obtained suspension with an ultrasonic cell disrupter until the solution is clear.
4. Then, centrifuge the homogenates for 5 minutes at 10000 × g and collect the supernatant and assay immediately or store in aliquots at ≤ -20℃.

Note: Tissue homogenates are recommended to be tested for protein concentration at the same time to obtain a more accurate concentration of the test substance per mg of protein.

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

1. Adherent cells should be washed by pre-cooled PBS gently, and then be detached with trypsin, and collect them by centrifugation at 1000 × g for 5 minutes (suspension cells can be collected by centrifugation directly).
2. Wash cells 3 times in pre-cooled PBS.
3. Then, resuspend the cells in fresh lysis buffer with concentration of 10^7 cells/mL. If it is necessary, the cells could be subjected to ultrasonication until the solution is clear.
4. Centrifuge at 1500 × g for 10 minutes at 2-8℃ to remove cellular debris. Assay immediately or store in aliquots at ≤ -20℃.

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℃. Avoid repeated freeze-thaw cycles.

Saliva - Collect saliva using a collection device or equivalent. Centrifuge samples at 1000 × g at 2-8℃ 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 50mg. The feces were washed three times with PBS (w:v = 1:9, e.g. 900μL lysis buffer is added in 100mg feces), sonicated (or mashed) and centrifuged at 5000×g for 10 minutes, where the supernatant was collected for testing.

Cell culture supernatants and other biological fluids - Centrifuge samples at 1000 × g for 20 minutes. Collect the supernatant and assay immediately or store samples in aliquot at -20℃ or -80℃ for later use. Avoid repeated freeze-thaw cycles.

Cerebrospinal fluid (CSF) - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

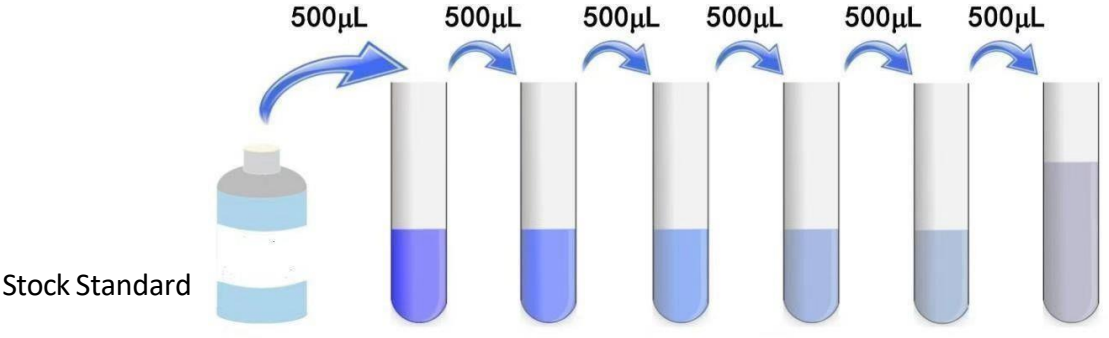
Notes

1. Samples to be used within 5 days may be stored at 4℃, otherwise samples must be stored at -20℃ (≤ 1 month) or -80℃ (≤ 2 months) to avoid loss of bioactivity and contamination. Avoid repeated freeze- thaw cycles.
2. Sample hemolysis will influence the result, so it should not be used.
3. When performing the assay, bring samples to room temperature.

Reagent Preparation

1. Bring all reagents to room temperature (18-25℃) before use. If the kit will not be used up in one assay, please only take out the necessary strips and reagents for present experiment, and store the remaining strips and reagents at required condition.
2. **Wash Buffer:** Dilute 30mL of Concentrated Wash Buffer with 720mL of deionized or distilled water to prepare 750mL of Wash Buffer.

   Note: if crystals have formed in the concentrate, warm it in a 40℃ water bath and mix it gently until the crystals have completely dissolved.
3. **Standard working solution:** Centrifuge the Reference Standard at 10000 × g for 1 minute. Add 1mL of Reference Standard & Sample Diluent, let it stand for 10 minutes and then mix gently, that is 5000pg/mL of standard working solution. Prepare 7 tubes containing 0.5mL Standard & Sample Diluent, to each tube, take 0.5mL from 5000pg/mL to make 2500pg/mL, and so on, the last is blank (no more liquid need to be drawn from the penultimate tube). Use it now.



4. **Biotinylated Detection Ab working solution:** Calculate the required amount before the experiment (50µL/well). In preparation, slightly more than calculated should be prepared. Centrifuge the stock tube before use, dilute the 100× Concentrated Biotinylated Detection Ab to 1× working solution with Biotinylated Detection Ab Diluent.
5. **HRP Conjugate working solution:** Calculate the required amount before the experiment (100µL/well). In preparation, slightly more than calculated should be prepared. Centrifuge the stock tube before use, dilute the 100× Concentrated HRP Conjugate to 1× working solution with HRP Conjugate Diluent.

Experimental Operation Tips

1. Solutions should be added to the bottom of the micro ELISA plate well, avoid touching the inside wall and causing foaming as much as possible.
2. Make the tested strips in use immediately after the wash step. Do not allow wells to be dry.
3. After adding the Substrate Reagent. The reaction time can be shortened or extended according to the actual colour change, but not more than 30 minutes.
4. Adding the stop solution should be done in the same order as the substrate solution.

Notes

1. The detection range of the kit is different from the concentration range of the substance to be measured in the sample. It is recommended to estimate the concentration by looking up the reference before the experiment, and determine the actual concentration by pre-experiment. If the concentration is too high or too low, dilute or concentrate the sample appropriately.
2. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
3. Some recombinant protein may not be detected due to a mismatching with the coated antibody or detection antibody.
4. If a lysis buffer is used to prepare tissue homogenates or cell culture supernatant, there is a possibility to cause a deviation due to the introduced chemical substance.

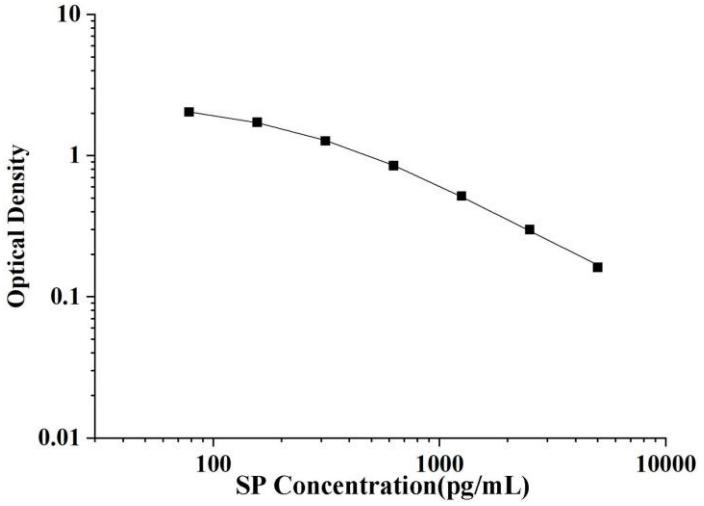
5. Samples should be used within 6 days when stored at 2-8℃, otherwise samples must be stored at -20℃ (≤1 month) or -80℃ (≤ 3 months). Avoid repeated freeze-thaw cycles.
6. With the standard product concentration as the horizontal coordinate and OD value as the vertical coordinate, the standard curve is formed by using the nonlinear four parameter logistic(4-PL) and the sample concentration is calculated by the curve.

Assay Procedure

1. Determine wells for diluted standard, blank and sample. Add 50µL each dilution of standard, blank and sample into the appropriate wells.
2. Immediately add 50µL of Biotinylated Detection Ab working solution to each well. Cover the plate with a new sealer. Incubate for 30 minutes at 37°C.
3. Decant the solution from each well add 300µL of wash buffer to each well. Soak for 30 seconds and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times.
4. Add 100µL of HRP Conjugate working solution to each well. Cover the plate with a new sealer. Incubate for 30 minutes at 37℃.
5. Decant the solution from each well, repeat the wash process for 3 times as conducted in step 3.
6. Add 100µL of Substrate Reagent to each well. Cover the plate with a new sealer. Incubate for about 15 minutes at 37°C. Protect the plate from light.
7. Add 50µL of Stop Solution to each well.
8. Determine the optical density (OD value) of each well at once with a micro plate reader set to 450nm.

Calculation of Results

For each test, a standard curve must be set for each plate. The standard curves below are for example purposes only.



Note: this graph is for reference only

Sensitivity

The minimum detection concentration of the Dog SP detected by this kit is usually less than the 46.88pg/mL. The sensitivity was determined by adding two standard deviations to the mean OD value of twenty zero standard replicates and calculating the corresponding concentration.

Precision

Mean coefficient of variation for Intra-Assay and Inter-Assay: 3 samples with low, middle and high level concentration were tested for repeat multiple times, respectively. The results showed that the coefficient of variation of the kits was less than 10%, which met the precision quality control standard.

Specificity

This method has high sensitivity and specificity for the detection of Dog SP. No significant cross- reactivity was observed between the Dog SP and its analogues.

Recovery

The known concentration of Dog SP was added to different samples respectively, and the recovery experiment was conducted. The results showed that the recovery range and average recovery rate of the kit were 80-120%, which met the recovery quality control standard.

Linearity

Dilute linear experiments were performed on samples with Dog SP to evaluate the linearity of the kit. The results showed that the kit linear range (%) was 80-120%, which met the linear quality control standard.

Limitations of the Procedure

1. This kit is for laboratory scientific research only, we will not be responsible for any consequences if this kit is used for clinical diagnosis or any other procedures.
2. Due to the uncertainty of its validity, this kit may not be suitable for testing some special experimental samples, such as gene knockout experiments.
3. This kit should be used before its expiration date, and please strictly follow the instructions for storage.
4. Different manufacturers' kits or testing the same analyte by other methods may produce inconsistent results because we do not compare our products with those of other manufacturers.
5. Since the antibodies used in the kit are usually prepared from recombinant proteins as immunogen, and recombinant proteins can be limited by different fragmentation, expression and purification systems, it is not recommended to use this kit to detect recombinant proteins.
6. In order to get the best experimental results, please use only the reagents provided by the manufacturer, and do not mix reagents from different batches.
7. Due to the existing conditions and the limitations of science and technology, we cannot fully identify and analyze the raw materials provided by the supplier comprehensively. Therefore, the kit may have some quality and technical risks.
8. The possibility of interference cannot be excluded before all factors are tested in the ELISA immunoassay.
9. In order to obtain reproducible results, each step in the experiment should be controlled and variations in sample collection, handling and storage may also lead to differences in sample measurements.
10. Although each kit passes rigorous quality testing, differences in measured values between batches of kits can still be caused by factors such as shipping conditions and different laboratory equipment.

Troubleshooting & Solutions

Problem	Possible Causes	Subsequent Actions
Poor standard curve	Improper standard dilution	Ensure that standards are dissolved and diluted in the recommended manner
	Inaccurate pipetting	Periodically calibrate pipettes and check the pipette tips
	Evaporate the reaction solution	Seal the enzyme plate with plate sealer
	Incomplete plate washing	Adequate washing times and the amount of washing solution added
	Foreign matter in the bottom of wells	Clean the bottom of the plate before reading
Weak or no colour development	Insufficient reaction of reagents	Ensure incubation time and incubate at the recommended temperature
	Inadequate reagent volumes	Check the pipette and follow the steps strictly to operate
	Improper dilution	Check the reagent dilution process
	Inactivation of enzyme conjugate	Mix conjugate and substrate, check by colour development
	No stop solution added	Add appropriate amount of stop solution
	Waiting too long time to read	Read the plate in time
High background	Contaminated chromogenic solution	Replace chromogenic solution
	Colouring time is too long	Control the colouring time
	Wrong dilution of reaction reagent	Use the recommended dilution
	Inadequate washing of the plate	Adequate washing times and the amount of washing solution added