Canine N-Terminal pro-Brain Natriuretic Peptide (NT-ProBNP) ELISA Kit

ZellBio GmbH (Germany)

Cat. No：ZB-10109C-Ca9648

Samples Type Validated
Serum, Plasma, Cell Lysate, Cell Culture Supernatant and Other Related Biological Liquids.

For Research Use Only. Not For In Vitro Diagnosis

Please read this insert completely prior to using the product.

Important note:

Only the brochure inside the kit should be considered for the assay procedure, as it is the latest version of the insert.

ZellBio GmbH assay kit is used to **quantitative** assay **Canine N-Terminal pro-Brain Natriuretic Peptide (NT-ProBNP)** on the basis of the Biotin double antibody sandwich technology. **ZellBio GmbH** ELISA kit takes **one-step** method with which solutions need minimum diluting, because we simplify the dilute process by our lab techniques. This kit is for research only and is not for use in diagnostic procedures.

Intended Use

ZellBio GmbH Canine N-Terminal pro-Brain Natriuretic Peptide (NT-ProBNP) in the sample of Canine's **serum, plasma, cell lysate and cell culture supernatants** and also other related biological fluids.

Test principle

ZellBio GmbH assay kit uses enzyme-linked immunosorbent assay (ELISA) based on the Biotin double antibody sandwich technology to assay the **Canine N-Terminal pro-Brain Natriuretic Peptide (NT-ProBNP).** Add NT-ProBNP protein to the wells, which are pre-coated with anti-NT-ProBNP monoclonal antibodies and after that, add anti-NT-ProBNP antibodies labeled with biotin to combine with

streptavidin-HRP, which forms an immune complex. Remove unbound enzymes after incubation and washing. Add substrate. Then the solution will turn blue and change into yellow with the effect of acid. The shades of solution and the concentration of **Canine N-Terminal pro-Brain Natriuretic Peptide (NT-ProBNP)** are positively correlated.

Materials supplied in the Test Kit

	Materials	96 Tests	48 Tests
1	Standard (4000ng/L)	0.35ml	0.35ml
2	Standard diluent	3ml	3ml
3	Microplate	12×8	6×8
4	Strp-HRP-Conjugate Reagent	6ml	3ml
5	Wash Solution (30X)	20ml	20ml
6	Biotin-NT-ProBNP-Ab	1ml	0.5ml
7	Chromogen Solution	12ml	6ml
8	Stop Solution	6ml	3ml
9	Instruction	1	1
10	Adhesive Sheet	2	2

Materials required but not supplied

1. 37℃ incubator
2. Standard Microplate reader
3. Precision pipettes and Disposable pipette tips
4. Distilled water
5. Disposable tubes for Standards and samples dilution
6. Absorbent paper

Important Notes

1. Before opening the kit kept at the temperature of 2-8℃, it takes at least 30 minutes to increase naturally to room temperature. After breaking the seal of ELISA coated-plate, some of the stripes used should be kept in a hermetic bag.
2. When adding samples, sample injector must be used for each time and should also be frequently checked for its precision to avoid individual error.
3. The instruction must be strictly followed while the reading of ELISA reader must be set as the standard of determining the experiment result.
4. Pipette tips and seal plate membrane in hand should not be used more than once in order to avoid cross contamination.
5. All samples, washing concentration and wastes of every kind should be disposed of as an infective agent.
6. Other reagents not needed must be packed or covered. Reagents of different batches must not be mixed and should be used before their respective validity dates.

7. Substrate is sensitive to light and therefore should not be exposed to light for too long.

Washing method

Preparation of 1X Wash Buffer: Dilute 30X wash buffer with distilled water. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.

Manual washing method: Washing by hand: Shake off the liquids in the wells of the ELISA plate; Lay several bibulous papers on the test bed and pat hard the ELISA plate several times downward; then inject at least 300μl of diluted washing concentration for 1-2 minutes' soaking. Repeat this process as needed.

Automatic washing method: Washing by automatic plate washer: If there is an automatic plate washer, it should only be used in the test when you are quite familiar with its functions.

Assay Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level **Canine NT-ProBNP** were tested 12 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level **Canine NT-ProBNP** were tested on 3 different plates, 10 replicates in each plate.

CV (%) = (SD/mean) ×100

Intra-Assay: CV<10%

Inter-Assay: CV<12%

Specimen requirements

1. Samples containing NaN_3 must **not be tested** as it inhibits the activity of Horseradish Peroxidase (HRP).

2. After collecting the sample, extraction should be immediately carried out in accordance with related documents. **After extraction, experiment should be conducted immediately as well (during 24 hours, can be kept at 2-8℃). Keep the sample at -20℃ or deeper Freeze for a long time. Avoid repeated freeze-thaw cycles.**

3. **Serum**: Allow the blood to clot for 10-15 minutes at room temperature. Centrifuge it (at 2000-3000 RPM) for 10 minutes. Collect the supernatants carefully. When sediments occur during storage, centrifugation should be performed again.

4. **Blood plasma**: In accordance with the requirements of sample collection, EDTA or sodium citrate should be used as anticoagulation. Add EDTA or sodium citrate and mix them completely for minutes. Centrifuge it (at 2000-3000 RPM) for approximately 10 minutes. Collect the supernatants carefully. When sediments occur during storage, centrifugation should be performed again.

5. **Cell culture supernatant**: Collect by sterile tubes when examining secret components. Centrifuge (at 2000-3000 RPM) for approximately

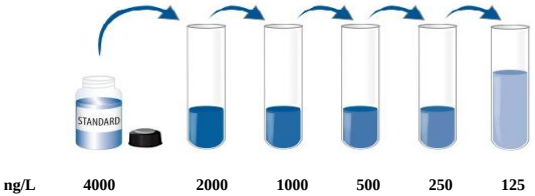
10 minutes. Collect the supernatants carefully. When examining the components within the cell, use PBS (pH 7.2-7.4) to dilute cell suspension to the cell concentration of approximately 1 million/ml. Damage cells through repeated freeze-thaw cycles to let out the inside components. Centrifuge (at 2000-3000 RPM) for approximately 10 minutes. Collect the supernatants carefully. When sediments occur during storage, centrifugation should be performed again.

6. **Solid Tissue sample**: Incise sample and weigh up. Add a certain amount of PBS (pH 7.4, 100mM) as a common suggestion buffer. Or freeze the tissue with liquid nitrogen immediately for later use. Thaw the sample and keep it at 2-8℃. Add PBS and homogenize the sample thoroughly by hand or homogenizer (totally **100mg tissue/1ml buffer**). Note: The PBS must contain **anti-protease cocktail** for preventing protein destruction. Centrifuge (at 4000-6000 RPM) for approximately 10 minutes. Collect the supernatants carefully. Aliquot and keep one for examination and freeze the others for later use. **After collecting the supernatant, experiment should be conducted immediately or the sample kept at 2-8℃ for 24 hours. Keep the sample at -20℃ or deeper Freeze for a long time. Avoid repeated freeze-thaw cycles.**

Assay procedure

1. Dilution of standard solutions: This kit has a standard of original concentration, which could be diluted in small tubes by user independently following the instruction:

4000ng/L	Standard No.6	60μl Original Standard
2000ng/L	Standard No.5	60μl Original Standard + 60μl Standard diluents
1000ng/L	Standard No.4	60μl Standard No.5 + 60μl Standard diluents
500ng/L	Standard No.3	60μl Standard No.4 + 60μl Standard diluent
250ng/L	Standard No.2	60μl Standard No.3 + 60μl Standard diluent
125ng/L	Standard No.1	60μl Standard No.2 + 60μl Standard diluent
0ng/L	Standard No.0	60μl Standard diluent



2. The number of stripes needed is determined by that of samples to be tested and added by that of standards. It is suggested that each standard solution and the blank well should be arranged with duplicates as much as possible.

3. Sample injection: a) Blank well: no sample, anti NT-ProBNP antibody labeled with biotin or streptavidin-HRP is added to

comparison blank well except Chromogen solution and stop solution while taking the same steps that follow. b) Standard solution well: Add 50μl standard and streptavidin-HRP 50μl (biotin antibodies have united in advance in the standard so no biotin antibodies are added). c) Sample well to be tested: Add 40μl sample and then 10μl NT-ProBNP antibodies, 50μl streptavidin-HRP. Then cover it with a seal plate membrane. Shake gently to mix them up. Incubate at 37℃ for 60 minutes.

4. Preparation of washing solution: Dilute the washing concentration (30X) with distilled water for later use.

5. Washing: Remove the seal plate membrane carefully, drain the liquid and shake off the remaining liquid. Fill each well with a washing solution. Drain the liquid after 30 seconds' standing. Then repeat this procedure four times and blot the plate.

6. Color development: Add 100μl Chromogen solution to each well as well. Incubate for 10-20 minutes at 37℃ away from light for color development.

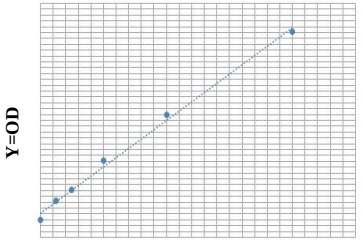
7. Stop: Add 50μl Stop Solution to each well to stop the reaction (the blue color changes into yellow immediately at that moment).

8. Reading: Take a blank well as zero, measure the absorbance (OD) of each well one by one under 450 nm wavelength, which should be carried out within the 10 minutes after having added the stop solution.

9. According to standards' concentrations and the corresponding OD values, Draw the standard curve using point to point calculation mode. Then according to the OD value of samples, calculate the concentration of the corresponding sample. Special software could be employed to calculate as well.

Calculation

Make concentration of standards the abscissa and OD value the ordinate. Draw the standard curve on the coordinate paper. According to the OD value of the sample, locate its corresponding concentration; point to point or calculate the linear regression equation of standard curve according to the concentration of the standards and the OD value. Then substitute with the OD value of the samples to calculate their concentration.



X= Canine NT-ProBNP Concentration

A typical standard curve of ZellBio GmbH **Canine NT-ProBNP** Assay kit
(The chart is indicative only)

Assay range：125ng/L →4000ng/L

Sensitivity：15ng/L

Summary

Prepare reagents, samples and the standards

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Add (40μl sample(s) +10μl NT-ProBNP-Ab), 50μl standards and 50μl Streptavidin-HRP, Let them react for 60 minutes at 37℃

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Wash the plate five times with 300μl diluted wash buffer

⇓

Add 100μl Chromogen solution. Incubate for 10-20 minutes at 37℃ for color development

⇓

Add 50μl stop solution

⇓

Read the OD value within 10min at 450 nm

⇓

Calculation

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