BT LAB
Bioassay Technology Laboratory

Optimize Your Research

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Canine Melatonin ELISA Kit

USER INSTRUCTION

Cat.No BTB-E0406Ca

Standard Curve Range: 5-1000ng/L

Sensitivity: 2.66ng/L

Size: 96 wells / 48 wells

Storage: Store the reagents at 2-8°C. For over 6-month storage refer to the expiration date keep it at -20°C. Avoid repeated thaw cycles. If individual reagents are opened it is recommended that the kit be used within 1 month.

***This product is for research use only, not for use in diagnosis procedures. It's highly recommended to read this instruction entirely before use.**

Precision

Intra-Assay Precision (Precision within an assay) Three samples of known concentration were tested on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision between assays) Three samples of known concentration were tested in separate assays to assess inter-assay precision.

CV(%) = SD/mean x 100

Intra-Assay: CV<8%

Inter-Assay: CV<10%

Intended Use

This sandwich kit is for the accurate quantitative detection of Canine Melatonin (also known as MT) in serum, plasma, cell culture supernates, Ascites, tissue homogenates or other biological fluids.

Assay Principle

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Canine MT antibody. MT present in the sample is added and binds to antibodies coated on the wells. And then biotinylated Canine MT Antibody is added and binds to MT in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated MT antibody. After incubation unbound

Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of Canine MT. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

Reagent Provided

Components	Quantity (96T)	Quantity (48T)
Standard Solution (1280ng/L)	0.5ml x1	0.5ml x1
Pre-coated ELISA Plate	12 * 8 well strips x1	12 * 4 well strips x1
Standard Diluent	3ml x1	3ml x1
Streptavidin-HRP	6ml x1	3ml x1
Stop Solution	6ml x1	3ml x1
Substrate Solution A	6ml x1	3ml x1
Substrate Solution B	6ml x1	3ml x1
Wash Buffer Concentrate (25x)	20ml x1	20ml x1
Biotinylated Canine MT Antibody	1ml x1	1ml x1
User Instruction	1	1
Plate Sealer	2 pics	2 pics
Zipper bag	1 pic	1 pic

Material Required But Not Supplied

- 37°C±0.5°C incubator
- Absorbent paper
- Precision pipettes and disposable pipette tips
- Clean tubes
- Deionized or distilled water
- Microplate reader with 450 ± 10nm wavelength filter

Precautions

- Prior to use, the kit and sample should be warmed naturally to room temperature 30 minutes.
- This instruction must be strictly followed in the experiment.
- Once the desired number of strips has been removed, immediately reseal the bag to protect the remain from deterioration. Cover all reagents when not in use.
- Make sure pipetting order and rate of addition from well-to-well when pipetting reagents.
- Pipette tips and plate sealer in hand should be clean and disposable to avoid cross-contamination.

- Avoid using the reagents from different batches together.
- Substrate solution B is sensitive to light, don't expose substrate solution B to light for a long time.
- Stop solution contains acid. Please wear eye, hand and skin protection when using this material. Avoid contact of skin or mucous membranes with kit reagent.
- The kit should not be used beyond the expiration date.

Specimen Collection

Serum Allow serum to clot for 10-20 minutes at room temperature. Centrifuge at 2000-3000 RPM for 20 minutes. Collect the supernatant without sediment.

Plasma Collect plasma using EDTA or heparin as an anticoagulant.After mix 10-20 minutes, centrifuge samples for 20 minutes at 2000-3000 RPM. Collect the supernatant without sediment.

Urine/Ascites/ Cerebrospinal fluid Collect by sterile tube. Centrifuge at 2000-3000 RPM for 20 minutes. Collect the supernatant without sediment.

Cell culture supernatant Collect by sterile tubes. When detecting secrete components, centrifuge at 2000-3000 RPM for 20 minutes. Collect the supernatants. When detecting the components in the cell, use PBS (pH 7.2-7.4) to dilute cell suspension , 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 20 minutes. Collect the supernatant without sediment.

Tissue Rinse tissues in ice-cold PBS (pH 7.4) to remove excess blood thoroughly and weigh before homogenization. Mince tissues and homogenize them in PBS (tissue weight (g): PBS (mL) volume=1:9) with a glass homogenizer on ice. To further break down the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 5 minutes at 5000×g to get the supernatant.

Note

- Sample concentrations should be predicted before being used in the assay. If the sample concentration is not within the range of the standard curve, users must **contact us** to determine the optimal sample for their particular experiments.
- Samples to be used within 5 days should be stored at 2-8°C. Samples should be aliquoted or must be stored at -20°C within 1 month or -80°C within 6 months. Avoid repeated freeze thaw cycles.
- Samples should be brought to room temperature before starting the assay.
- Centrifuge to collect sample before use.
- Samples containing NaN3 can't be tested as it inhibits the activity of Horse Radish Peroxidase (HRP).

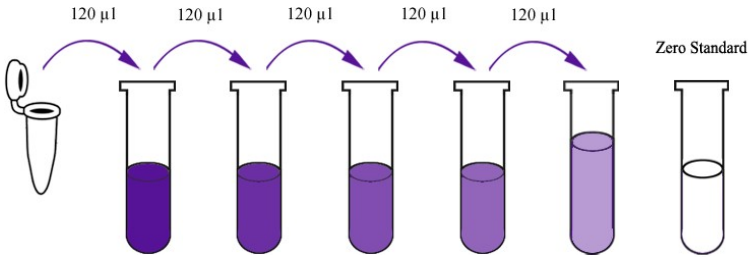
- Collect the supernatants carefully. When sediments occurred during storage, centrifugation should be performed again.
- Hemolysis can greatly impact the validity of test results. Take care to minimize hemolysis.

Sample can't be diluted with this kit. Owing to the the material we use to prepare the kit, the sample matrix interference may falsely depress the specificity and accuracy of the assay.

Reagent Preparation

- All reagents should be brought to room temperature before use.
- **Standard** Reconstitute the 120µl of the standard (1280ng/L) with 120µl of standard diluent to generate a 640ng/L standard stock solution. Allow the standard to sit for 15 mins with gentle agitation prior to making dilutions. Prepare duplicate standard points by serially diluting the standard stock solution (640ng/L) 1:2 with standard diluent to produce 320ng/L, 160ng/L, 80ng/L and 40ng/L solutions. Standard diluent serves as the zero standard(0 ng/L). Any remaining solution should be frozen at -20°C and used within one month. Dilution of standard solutions suggested are as follows:

640ng/L	Standard No.5	120µl Original Standard + 120µl Standard Diluent
320ng/L	Standard No.4	120µl Standard No.5 + 120µl Standard Diluent
160ng/L	Standard No.3	120µl Standard No.4 + 120µl Standard Diluent
80ng/L	Standard No.2	120µl Standard No.3 + 120µl Standard Diluent
40ng/L	Standard No.1	120µl Standard No.2 + 120µl Standard Diluent



Standard Concentration	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
1280ng/L	640ng/L	320ng/L	160ng/L	80ng/L	40ng/L

- **Wash Buffer** Dilute 20ml of Wash Buffer Concentrate 25x into deionized or distilled water to yield 500 ml of 1x Wash Buffer. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.

Assay Procedure

1. Prepare all reagents, standard solutions and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature.
2. Determine the number of strips required for the assay. Insert the strips in the frames for use. The unused strips should be stored at 2-8°C.
3. Add 50µl standard to standard well. **Note**: Don't add biotinylated antibody to standard well because the standard solution contains biotinylated antibody.
4. Add 40µl sample to sample wells and then add 10µl anti-MT antibody to sample wells, then add 50µl streptavidin-HRP to sample wells and standard wells (Not blank control well). Mix well. Cover the plate with a sealer. Incubate 60 minutes at 37°C.
5. Remove the sealer and wash the plate 5 times with wash buffer. Soak wells with 300ul wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirate or decant each well and wash 5 times with wash buffer. Blot the plate onto paper towels or other absorbent material.
6. Add 50µl substrate solution A to each well and then add 50µl substrate solution B to each well. Incubate plate covered with a new sealer for 10 minutes at 37°C in the dark.
7. Add 50µl Stop Solution to each well, the blue color will change into yellow immediately.
8. Determine the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

Summary

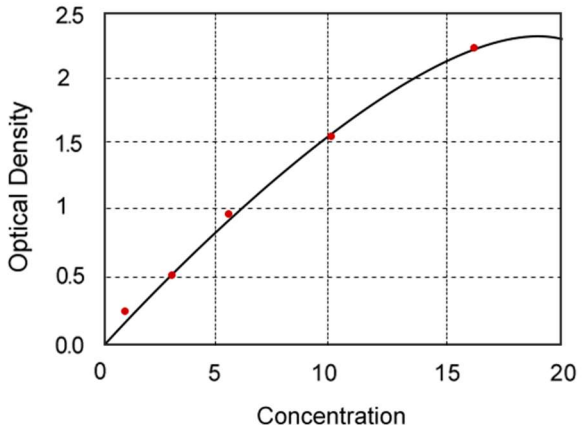
1. Prepare all reagents, samples and standards.
2. Add sample and ELISA reagent into each well. Incubate for 1 hour at 37°C.
3. Wash the plate 5 times.
4. Add substrate solution A and B. Incubate for 10 minutes at 37°C.
5. Add stop solution and color develops.
6. Read the OD value within 10 minutes.

Calculation of Result

Construct a standard curve by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and draw a best fit curve through the points on the graph. These calculations can be best performed with computer-based curve-fitting software and the best fit line can be determined by regression analysis.

Typical Data

This standard curve is only for demonstration purposes. A standard curve should be generated with each assay.



Troubleshooting

Possible Case	Solution
High Background	
• Improper washing	• Increasing duration of soaking steps
• Substrate was contaminated	• Replace. Substrate should be clean and avoid crossed contamination by using the sealer
• Non-specific binding of antibody	• Replace another purified antibody or blocking buffer
• Plate are not be sealing incompletely	• Make sure to follow the instruction strictly
• Incorrect incubation temperature	• Incubate at room temperature
• Substrate exposed to light prior to use	• Keep substrate in a dark place
• Contaminated wash buffer	• Use a clean buffers and sterile filter
Weak Signal	
• Improper washing	• Increasing duration of soaking steps
• Incorrect incubation temperature	• Incubate at room temperature
• Antibody are not enough	• Increase the concentration of the antibody
• Reagent are contaminated	• Use new one
• Pipette are not clean	• Pipette should be clean
No Signal	
• Reagent are contaminated	• Use new one
• Sample prepared incorrectly	• Make sure the sample workable/dilution
• Antibody are not enough	• Increase the antibody concentration
• Wash buffer contains sodium azide	• Use a new wash buffer and avoid sodium azide in it
• HRP was not added	• Add HRP according to the instruction
Poor Precision	
• Imprecise/ inaccurate pipetting	• Check/ calibrate pipettes
• Incomplete washing of the wells	Make sure wells are washed adequately by filling the wells with wash buffer and all residual antibody solutions crossed well before washing.