VERSION 1.01

ECHINOCOCCOSIS DOG FECES ANTIGEN ELISA KIT

MANUAL

ELISAKITS.ONLINE

By Immunomart

Echinococcosis Dog Feces Antigen ELISA Kit

Catalogue Number. IP100198

Product Usage

This test kit is used to detect Echinococcosis antigen in dog feces, used to help diagnosis for infection of Echinococcus granulosus in dogs and carnivores.
This test kit use double-antibody sandwich method, monoclonal antibody against Echinococcus granulosus is precoated on microplate. When run the test, add sample, then incubate, if there is Echinococcosis antigen in the sample, it will combine with the pre-coated monoclonal antibody; discard the uncombined antigen after washing, then add enzyme conjugate, forming pre-coated monoclonal antibody-antigen combination forming monoclonal antibody-enzyme conjugate complex; discard the uncombined enzyme conjugate; add substrate, the blue signal formed by the enzyme is proportional to the antigen content in the sample, measure the OD value by ELISA reader with wavelength at 450nm.

Specifications: 96 wells × 2.

Components

	Code item	**Spec.**
1	Echinococcus monoclonal antibody Coated plate	1 plate
2	Enzyme Conjugate	11 ml
3	10X Concentrated washing buffer	100 ml
4	Substrate	11 ml
5	5X Sample treatment solution	50 ml
6	Stop solution	11 ml
7	Negative control	1 ml
8	Positive control	1 ml
9	Adhesive Foil	2 piece
10	Instruction sheet	1 piece

Material required not provided

1. Micropipettes: 0.5μL~10μL、10μL~100μL、100μL~1000μL
2. Disposable pipette tips
3. Measuring cylinder: 500 ml
4. 96 wells microplate reader
5. Distilled water or deionized water
6. Bottle or microplate washing machine

Sample preparation

Take animal whole blood, get serum by using regular method, the serum should bright and no hemolysis

Return 10X Concentrated washing buffer into room temperature before use, if there is salt crystals, shake to make it dissolved, then dilute it at 10 times with distilled water or deionized water. The diluted washing buffer can store at 4℃ for about 1 week.

2) Return 5X Sample treatment solution into room temperature before use, if there is salt crystals, shake to make it dissolved, then dilute it at 5 times with distilled water or deionized water. The diluted Sample treatment solution can store at 4℃ for about 1 week.

Sample treatment:

Take 1.0 g dog feces sample into a centrifuge tube, add 2.0ml diluted Sample treatment solution, mix it evenly, centrifuge at 2000g for 10min, take up-layer clear liquid for test.

Note: negative and positive control do not need dilute

Notes

1) Return all reagents into room temperature before use, put all reagents at room temperature for at least 1 hour. Shake it evenly before use, and store back to 2-8℃ after usage.

2) Do not mix use reagents from different kits and different lot no., prevent the reagents been polluted when using.

3) Substrate A and stop solution may have irritation to skin and eyes, be careful to use.

4) Do not expose Substrate to strong light and avoid contact with the oxidant.

5) Antibody coated plates should be sealed and moisture-proof. Put back unused MicroWell plate into dry foil bag and sealed at 2~8 ℃.

6) All wastes should be treated well to avoid pollution before discarding.

7) Strict compliance with the operating instructions can get the best results. Pipetting operation, timing, and washing of the whole process must be precise.

Operation procedures

1 .Adding sample: Take out the required coated plates according to sample quantity (Can be detached) and record the sample position on a worksheet. For single-wave length test, set one blank control well, add nothing; for double-wave length test, do not set blank control well. Set 2 wells for negative control serum and 2 wells for positive control serum, add undiluted negative and positive control serum to its well accordingly, 100 µL/well. Others are wells for samples, add 100µL/well of Sample diluent solution, then add 1 µL serum sample separately (there will be color change after adding sample).

2. Mix gently for 30s, incubate at 37℃ for 30 min.

3. Remove adhesive foil. Pour the liquid out of the wells, add Washing solution(dillute the Wash Concentrate at 20 times with deionized water) into each well fully, stand for 1 min. Repeat 5 times, at last time pat to dry on absorbent paper.

4. Add 100 µL enzyme comjugate into each well (except the blank well, also do not add any liquid to blank well).

5. Cover plate with new adhesive foil. Incubate at 37 ℃ for 30 min.

6. Repeat step 3(washing).

7. Add 50µL substrate A and substrate 50µL B into each well, mix properly, incubate for 15 min at 37 ℃ in the dark with new adhesive foil.

8. Add 50µL stop solution into each well, mix gently and determine the result within 5-30 min.

9. For single-wavelength test, measure the A value with a photometer at 450 nm, set zero for the blank well, and read A value of each well; For double-wavelength test, measure the A value with a photometer at 450 nm (Reference-wavelength: 630 nm), read A value of each well.

Results

Read the OD value with ELISA reader at 450nm(630nm as reference)
For the assay to be valid:
OD Value of Negative control (N)< 0.2, meanwhile average OD value of Positive control (P)>0.4.
Calculation method:
Sample OD value / Positive control OD Average value= S/P value
The result is judged by S/P value:
If S/P≥0.5, it is positive; If S/P< 0.5, it is negative.

Notes

All reagents should be adjusted to the room temperature and shake evenly before using, store at 2-8 ℃ after using
2) Do not exchange the reagents from the kits of different lot numbers to use. Avoid reagent pollution when using.
3) Substrate and stop solution may have excitant to skin and eyes, pay attention when using.
4) Do not expose TMB (Substrate B) to light and avoid it contact with antioxidants.
5) The wells should avoid damp or touching water after unsealing (Put the un-using microplate back to bag with dehydrator in 2~8 ℃ soon)
6) Deal all waste reasonable before dumping to avoid pollution.
7) Strictly adhere to instruction to get best result. All procedure including pipetting, timing and washing etc. must be accurate.
8) Serum dilution plate is disposable, do not use for second time; the MAX volume of it is 300μL/well.

storage: store at 2-8℃, dark, sealed, dry place, no frozen..
Expiry date: 12 months; date of production is on box.