Echinococcus IgG GENLISA™ ELISA

REF : KBD120

Ver 1.1

IVD

Enzyme Immunoassay for Qualitative Determination of Echinococcus IgG in human serum and plasma.

IVD	**For In-vitro Diagnostic Use**	**REF**	**Catalog Number**
	**Store At**	**LOT**	**Batch Code**
	**Manufactured By**		**Biological Risk**
	**Expiry Date**		**Consult Operating Instructions**

REF KBD120 Σ 96 tests

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Introduction:

Ecchinoccus spp. are 1-6 mm long cestode parasites with 3 to 5 segments. The two species E. granulosus and E. multilocularis are principally responsible for human disease. These species are etiologic agents of Hydatid disease a zoonotic infection of worldwide distribution in man and other intermediate hosts. The parasite mainly affects canines like wolves, dogs, foxes as its definitive host and man as intermediate host also infected coincidentally by contact with dog feces. The adult worms live in small intestine of definitive host and their mature proglottids containing numerous eggs are released into the environment through the host feces. If eggs are ingested by intermediate host like human, sheep, etc. they will hatch in small intestine and released oncospheres will penetrate into the lumen wall and entered into the mesenteric blood circulation. They carried throughout the body by blood circulation and mainly affect liver and lungs where they produce Hydatid cysts. The cysts also have been reported from other body organs like brain, bones, heart and other organs. Most of the patients are asymptomatic for years but symptoms of disease arise from compression of adjacent host structures due to gradually enlarging hydatid cyst. Echinococcosis is potentially dangerous disease of human and untreated cases are highly fatal. Nowadays various serological tests including IHA, IFA and ELISA have been used for serological diagnosis of hydatid disease. The degree of immune response not only affect by the parasite but other factors like cyst location and size also affect the degree of response. Studies have been shown that bone and liver cysts have higher antibody response than those located in lungs, spleen and brain.

Intended Use:

The Echinococcus IgG GENLISA™ ELISA is intended for the qualitative determination of IgG class antibodies in human serum and plasma.

Principle:

Echinococcus IgG GENLISA™ ELISA is an indirect enzyme linked immnunosorbent assay for qualitative determination of IgG antibody present in the human serum and plasma. Echinococcus Antigen is pre-coated onto microwells. Samples, Controls are pipetted into microwells and Echinococcus IgG antibody present in sample binds to the antigen coated on the wells. Enzyme Conjugate antibody is pipetted and incubated to form an immune complex. After washing microwells in order to remove any non-specific binding, the substrate solution is added to microwells and color develops proportionally to the amount of Echinococcus IgG present in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. Echinococcus granolusus antigens Coated Microtiter Plate (96 wells) - 1 no
2. Positive control – 1 ml
3. Negative control – 2 ml
4. Enzyme conjugate - 12 ml
5. Sample diluent – 2 x 50 ml
6. (20X) Wash Buffer – 50 ml
7. TMB Substrate - 12 ml
8. Stop Solution - 12 ml
9. Instruction Manual

Materials to be provided by the End-User:

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Deionized (DI) water
3. Precision single and multi-channel pipette and disposable tips.
4. Disposable pipette tips.
5. Absorbent paper.

Handling/Storage:

1. Kit should be stored at 2-8°C upon receipt and when it is not in use.

2. Keep un-used wells in their sealed bag with desiccants.
3. Do not use expired date reagents.
4. Do not freeze.
5. Protect from light and moisture

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.
2. For In-vitro use Only.

Specimen Collection and Handling:

Serum- Coagulate at room temperature for 10 - 20 minutes; centrifuge for 20 minutes at 2000-3000 rpm. Remove the supernatant. If precipitation appears, re-centrifuge.

Plasma- Use EDTA or citrate plasma as an anticoagulant, mix for 10 - 20 minutes; centrifuge for 15 minutes at 2000-3000 rpm. Remove the supernatant carefully. If precipitation appears, re-centrifuge.

Sample Dilution:
To make 1:101 Dilution, dilute 10 ul Sample + 1 ml Sample Diluent. Mix well.

Reagent Preparation:

1. Allow all components to reach RT (Room Temperature) prior to use in the assay.
2. (1X) Wash Buffer Dilution: To make (1X) Wash Buffer, add 50ml of (20X) Wash Buffer to 950ml of DI water. This is the working solution.

Test Procedure:

1. All reagents should be allowed to reach room temperature before use.
2. Add **100 ul Positive Control, Negative Control and Diluted Sample** in appropriate wells.
3. Seal the plate and Incubate at room temperature for 30 minutes.
4. Aspirate and wash plate 5 times with **(1X) Wash Buffer** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.
5. Add **100 ul** of **Enzyme Conjugate** to each well except blank well.
6. Incubate at room temperature for 30 minutes.
7. Repeat the Wash Step as mentioned in step 4.
8. Add **100 ul** of **TMB Substrate** into each well.
9. Incubate at room temperature in the dark for 15 minutes.
10. Add **100 ul** of **Stop Solution**. Read result with an ELISA reader at 450 nm within 15 minutes of stopping the reaction.

Interpretation of Results:

Determine the Mean Absorbance (net of Blank) for each set of duplicate Controls and Samples. Results are interpreted qualitatively by calculating a cut-off value for each sample on the basis of the cut-off determined. Read Absorbance at 450nm with an ELISA reader.

Cut-off = Mean OD of Negative Control + 0.25

Positive Results: **OD value > CO**
Those samples with OD values of higher than cut-off value must be considered as positive for specific anti-Echinococcus IgG antibody.

Negative Results: **OD value < CO**
Those specimens with OD values of lower than cut-off value should be considered as negative for specific anti-Echinococcus IgG antibody.

Those specimens with OD values of too close to cut off value should be re-tested with fresh specimen after a 2-4 weeks to rule out possible Echinococcus infection.

Criteria of Validation:

Blank	O.D < 0.1
Negative Control	O.D < 0.20
Positive Control	O.D > 0.6

Performance Characteristics:

Precision:
Results are shown in table 1 and 2:

Table1: Intra-assay

	No. of Tests	Mean OD	SD	CV %
Negative control	24	0.06	0.004	6.7
Positive control	24	2.35	0.074	3.1

Table 2: Inter-assay

	No. of Tests	Mean OD	SD	CV %
Negative control	10	0.07	0.0069	9.8
Positive control	10	2.41	0.108	4.5

*Each test has been run in duplicate

Sensitivity & Specificity:

A total of 36 patients suspected Echinococcus infection with clinical signs and symptoms related to Hydatid cyst disease were evaluated. Of these, 11 were confirmed positive and 25 were negative by commercial ELISA kit. The ELISA test results were compared to the commercial kits.

	Echinococcus IgG GENLISA™ ELISA			
Commercial ELISA kit		**Positive**	**Negative**	**Total**
	+	**10**	**1**	**11**
	-	**1**	**24**	**25**
Total				**36**

Relative Sensitivity = 10 / 11 = 91%
Relative Specificity = 24 / 25 = 96%
Relative Accuracy = 34 / 36 = 94%

Correlation Test:

175 patient sera were tested by Echinococcus IgG GENLISA™ ELISA kit and a reference ELISA kit. 5 sera were positive and 167 were negative by both methods (98% agreement).

		Echinococcus IgG GENLISA™ ELISA		
		+	-	Total
Reference ELISA kit	+	5	2	7
	-	1	167	168
	Total	6	169	175

Reproducibility:

It has been calculated on the negative and positive controls tested in replicates in different days. CV's between 3-10% have been obtained dependent on their OD values at 450 nm.

Safety Precautions:

- **This kit is For In-vitro Diagnostic Use only**. Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Some of the reagents contain small amount of sodium azide (< 0.1 % w/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials maybe derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed

- Do not smoke, eat or drink while handling kit material
- Always use protective gloves
- Never pipette material by mouth
- Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.

- In any case GLP should be applied with all general and individual regulations to the use of this kit.

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Regulatory Status:

CE Marked	Europe
FDA registered	USA
CDSCO registered	India

SCHEMATIC ASSAY PROCEDURE

1	All reagents should be allowed to reach room temperature before use.
2	Add **100 ul Positive Control, Negative Control and Diluted Sample** in appropriate wells.
3	Seal the plate and **Incubate at room temperature for 30 minutes.**
4	Aspirate and wash plate 5 times with (1X) Wash Buffer and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.
5	Add **100 ul of Enzyme Conjugate** to each well except blank well.
6	**Incubate at room temperature for 30 minutes.**
7	Repeat the Wash Step as mentioned in step 4.
8	Add **100 ul of TMB Substrate** into each well.
9	**Incubate at room temperature in dark for 10 minutes**.
10	Add **100 ul of Stop Solution**. Read result with an ELISA reader at 450 nm within 15 minutes stopping the reaction.

SYMBOLS KEY

MTP	Coated Microtiter Plate (8x12 wells)
POS CNTRL	Positive Control
NEG CNTRL	Negative Control
ENZY CONJ	Enzyme Conjugate
SAMP DIL	Sample Diluent
20X WASH BUF	(20X) Wash Buffer
SUB TMB	TMB Substrate
SOLN STOP	Stop Solution
	Consult Instructions for Use
REF	Catalog Number
	Expiration Date
	Storage Temperature