

## ***Fasciola hepatica*** **IgG ELISA Kit**

96 Tests Kit

Enzyme Immunoassay for the  
Determination of *Fasciola hepatica* IgG level  
in Human Serum/Plasma  
(For In Vitro Diagnostic Use Only)  
Catalogue No. PT-Fas-96

**PISHTAZ TEB DIAGNOSTICS**

### **Introduction**

*Fasciola hepatica* is a trematode parasite which affects livestock like cattle, sheep, etc. Human infection is reported to be progressively increased since 1980 (WHO report). The parasite prevalent in most regions of the world and epidemics are reported from various countries. Fascioliasis disease complications are mainly due to parasite migration to the liver and biliary tract and production of damage to the liver hepatocytes. In bile ducts, presence of adult worms cause mechanical and toxic damage to the biliary tract and in late stages of disease, superimposed secondary bacterial infection leads to cirrhosis in affected patients. Clinical manifestations of disease in human are mainly: liver spasms or cramps (RUQ pain), nausea and vomiting, abdominal pain, headache, irregular fever with marked sweating, diarrhea, anemia and eosinophilia in peripheral blood examination. The only confirmatory test for diagnosis of fascioliasis is finding the parasite eggs in feces or duodenal lavage of infected patients. However, other tests like complement fixation test, skin test and haemagglutination inhibition test are also used for the diagnosis of fascioliasis but they suffer from lower sensitivity. The above confirmatory test also has some limitation for example only after 7-11 weeks since the beginning of infection; it is possible to find parasite eggs in feces. It has been shown that patients ingesting animal liver containing parasite eggs produce false positive results in stool microscopic examination (pseudo fasciolosis).

One of the definitive tests for diagnosis of fascioliasis is ELISA test. The employment of ELISA test for the diagnosis of fascioliasis, make diagnosis possible 2-4 weeks of post infection. The sensitivity of ELISA is about 98% and a few cross reactions were reported with *Schistosoma* parasite.

### **Test Principle**

The test principle is based on indirect ELISA test. In this technique, microplate wells are coated with certain amount of *Fasciola hepatica* specific antigens. Diluted serum samples are added into the microplate wells and allowed to react with antigens immobilized on the solid phase. If specific antibodies were presented in sera they will bind to the solid phase *Fasciola hepatica* antigens. After washing the microplate wells and removing unbound antibodies, anti Human IgG- horseradish peroxidase (HRP) is added into the wells. The wells are washed again with washing solution to remove unbound labeled antibodies. A solution of chromogen-substrate is then added and incubated for 15 minutes, resulting in the development of a blue color. The color development is stopped by the addition of stop solution and measured spectrophotometrically at 450 nm.

### **Materials provided with the kit:**

1. *Fasciola hepatica* antigen coated wells ( 1 plate, 96 wells): Microtiter wells coated with *Fasciola hepatica* antigens.
2. Sample diluent (2 vials, each 50 ml): Contains phosphate buffer solution with protein as stabilizer and Kathon CG as preservative.
3. Enzyme conjugate (1 vial, 12 ml): Contains polyclonal anti human IgG labeled with HRP, ready to use
4. Negative control (2 ml / vial): Negative pooled sera in buffer containing protein as stabilizer and Kathon CG as preservative, ready to use.
5. Positive serum control (1 ml / vial): Positive pooled sera containing anti *Fasciola hepatica* antibodies diluted in buffer which contains protein as stabilizer and Kathon CG as preservative, ready to use.
6. Wash solution (1 vial, 50 ml): Contains phosphate buffer solution with 0.05 %



- tween 20 as detergent, pH=6, concentrated (20X).
7. Chromogen substrate reagent ( 1 vial, 12 ml): Contains tetramethylbenzidine and hydrogen peroxide, ready to use.
  8. Stop solution (1 vial / 12 ml): Contains 1 molar hydrochloric acid, pH< 1.
  9. Cardboard sealer.

testing. If prolong storage is required, samples should be stored at -20°C.  
Avoid freeze-thaw of specimen during storage.

### Materials required but not provided:

1. ELISA reader with 450 nm filter.
2. Precision micropipettes.
3. Distilled water.
4. Disposable pipette tips.
5. Absorbent paper or paper towel.
6. Graph paper.

### Reagents Preparation

1. All reagents should be allowed to reach room temperature (22-28°C) before use.
2. Working wash solution: dilute concentrated wash solution 1:20 with distilled water before use.
3. Dilute specimens 1:101 with sample diluent (i.e. 10 µl of specimen with 1 ml of sample diluent).

### General Information

1. Do not mix kit reagents from different batch/lot numbers. All kit components must be used only in their original kit.
2. All reagents obtained from human sources are negative for HBs Ag, anti-HCV and anti-HIV antibodies. To prevent risk of contamination, use personal protective equipments like gloves, lab coats, etc. and avoid direct contact with reagents.

### Assay Procedure

1. Secure the desired number of microplate wells in the holder and keep the remaining with desiccants in tightly closed special bag.
2. Dispense 100 µl of serum controls (the serum controls are ready to use) and diluted specimen samples in appropriate wells according to following order: (Table 1)

- Use the first well as blank (BL)
- Use the next two wells for negative control (NC).
- Use one well for the positive control. (PC)
- Use the remaining wells for specimens.

### Storage Conditions

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.

### Specimen Collection and Preparation

The kit is for use with serum or plasma. Serum or plasma should be prepared from a whole blood specimen obtained by approved aseptic technique. If testing cannot be done within an hour after sample collection, refrigerate (maximum 48 hours) the specimen immediately and let it return to room temperature before



Table no. 1: Samples order in an ELISA microplate

BL	T5	T13	T21	T29	T37	T45	T53	T61	T69	T77	T85
NC	T6	T14	T22	T30	T38	T46	T54	T62	T70	T78	T86
NC	T7	T15	T23	T31	T39	T47	T55	T63	T71	T79	T87
PC	T8	T16	T24	T32	T40	T48	T56	T64	T72	T80	T88
T1	T9	T17	T25	T33	T41	T49	T57	T65	T73	T81	T89
T2	T10	T18	T26	T34	T42	T50	T58	T66	T74	T82	T90
T3	T11	T19	T27	T35	T43	T51	T59	T67	T75	T83	T91
T4	T12	T20	T28	T36	T44	T52	T60	T68	T76	T84	T92

BL: Blank, NC: Negative control, PC: Positive control, T1-T92: Test 1-Test 92

### Validity of the Assay

The assay is to be considered valid if:

- Cover the microplate wells with cardboard sealer tightly. Incubate wells for 30 minutes at 22-28°C.
- Remove the incubation mixture by flicking plate contents into a waste container. Rinse and flick the microtiter wells 5 times (each with 300 µl of working wash solution).
- Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
- Add 100 µl of ready to use anti-Human IgG -HRP conjugate into the microplate wells except wells labeled "blank".
- Cover the plate with cardboard sealer tightly. Leave wells for 30 minutes at 22-28°C.
- Repeat step 4 and 5
- Dispense 100 µl of chromogen/substrate to each well.
- Incubate the microplate wells at room temperature and dark for 15 minutes to develop color.
- Add 100 µl of stop solution to the wells to stop reaction.
- Read absorbance at 450 nm by ELISA reader (Use 630 nm filter as reference filter if it's available)
- The OD 450 nm of the blanking well is lower than 0.1. Higher values indicate chromogen/substrate contamination. In such a case, repeat the assay carefully checking the reagent.
- After subtracting the blank, the mean OD value for the Negative control is lower than 0.20. Higher values indicate an incorrect washing procedure. In such a case, check the efficiency of the washing device.
- The value of positive control is higher than the 0.6 OD 450nm. Lower values indicate kit reagents decay. In such a case, check expiry date of the kit before repeating the assay.

### Result Calculation

- Measure absorbance of controls and samples at 450 nm (Use 630 nm filter as reference filter if it's available) Subtract OD value of blank from all controls and tests OD.
- To calculate cut off value for the test, use following formulae:

$$\text{Cut-off} = \text{N.C. mean OD at 450 nm} + 0.25$$

- Those samples with OD values of greater than cut-off value must be considered as positive for specific anti-*Fasciola hepatica* IgG antibody.



4. Those specimens with OD values of lower than cut-off value should be considered as negative for specific anti-*Fasciola hepatica* IgG antibody.

### Performance Characteristics

#### Sensitivity & Specificity

A total 134 patients, referred to physicians with clinical signs and symptoms related to Fasciolosis, were evaluated. Of these, 12 were confirmed positive and 122 were negative by coprology method (presence of *Fasciola* eggs in stool samples). The ELISA test results were compared to the coprologic method findings.

#### Pishtaz teb diagnostics *Fasciola* IgG ELISA kit

	Positive +	Equivocal	Negative -	Total
Coprology	+	-	1	12
	-	-	114	122
<b>Total Samples</b>				<b>134</b>

Sensitivity =  $11 / 12 = 92\%$   
 Specificity =  $114 / 122 = 93\%$   
 Accuracy =  $125 / 134 = 93\%$

#### Correlation Test

183 patient's sera were tested by Pishtazteb *Fasciola* IgG ELISA kit and a reference ELISA kit. 4 sera were positive and 175 were negative by both methods (97 % agreement).

		Pishtazteb <i>Fasciola</i> IgG ELISA kit		
		+	-	Total
Reference ELISA kit	+	4	2	6
	-	2	175	177
	Total	6	177	183

### 3. Reproducibility

It has been calculated on the negative and positive controls tested in replicates in different days. CV's between 4-10% have been obtained depend on their OD values at 450 nm.

Table no. 1 (Intra-assay)

	No. of Tests Performed	Means OD	SD OD	CV%
Negative control	24	0.055	0.003	5.4
Positive control	24	1.5	0.04	2.7

Table No. 2 (Inter-assay)

	No. of Tests Performed	Means OD	SD OD	CV%
Negative control	10	0.06	0.006	10
Positive control	10	1.49	0.06	4.0




\*Each test has been run in duplicate

#### References:

- 1 – Mascomas s, Bargues MD, Valero. (2005). Fascioliasis and other plant borne termatode zoonoses. *Int J Parasitol* 35: 1255 – 78.
- 2 – Liu LX, Harinasuta KT. (1996). Liver and intestinal flukes. *Gastroenterol Clin North Am* 25: 627 – 36.
- 3 – Garcia LS. (2001). *Diagnostic medical parasitology*, 4 th ed, American Society for Microbiology, Washington DC.



***Fasciola hepatica* IgG ELISA  
 Test Procedure**
**Step 1**

Reagent	Blank	Serum Control	Diluted Sample
			
Control Serum	None	100 µl	None
Diluted Sample	None	None	100 µl




Read absorbance at 450 nm (Use 630 nm filter as reference filter if it's available).

Cover the microplate wells with cardboard sealer tightly and incubate them for 30 minutes at room temperature.


**Step 2**

Remove plate cover and discard contents of the wells. Wash the microplate wells for 5 times according to test manual.






HRP conjugate	None	100 µl	100 µl
			

Cover the microplate wells with cardboard sealer and incubate for 30 minutes at RT.


**Step 3**

Remove plate cover and discard contents of the wells. Wash the microplate wells for 5 times according to test manual.

Chromogen-Substrate solution	100 µl	100 µl	100 µl
			

Incubate wells for 15 minutes at room temperature and dark.


**Step 4**

Stop Solution	100 µl	100 µl	100 µl
	