CE

AccuDiag™ Schistosoma IgG ELISA Kit

REF 8209-35

IVD See external Label 2°C 8°C 96 Tests

Test	Schistosoma IgG ELISA
Method	Enzyme Linked Immunosorbent Assay
Principle	Sandwich Complex
Detection Range	Qualitative : Positive, Negative
Sample	5 µL serum
Total Time	~ 25 min.
Shelf Life	12 Months from the manufacturing date
Specificity	85%
Sensitivity	100%

INTENDED USE

The Schistosoma ELISA Kit is For the qualitative determination of serum antibodies in humans, primarily IgG, to Schistosoma spp. using the ELISA technique.

SUMMARY AND EXPLANATION

Schistosomiasis is a disease caused by parasitic worms of the genus Schistosoma. People are at risk for Schistosomiasis infection when they are exposed to the parasites, normally by bathing or swimming in contaminated water. Infection is transmitted when larvae from the parasite (usually freshwater snails) penetrates the skin and travels through the bloodstream. Acute and chronic symptoms result when the egg migration of the worms affects vital tissue and organs. Some affected people may be asymptomatic, but generally symptoms range skin irritation, to fever, intestinal and urinary tract infections, to possibly life-threatening complications. The disease has affected more than 200 million people worldwide, and has been classified as the second most common tropical disease following Malaria.

TEST PRINCIPLE

During the first incubation, the antibodies in the patients' serum bind to the antigens in the test well. The next incubation allows the enzyme complex to bind to the antigen-antibody complex. After a few washings to remove unbound enzymes, a substrate is added that develops a blue color in the presence of the enzyme complex and peroxide. The stop solution ends the reaction, turning the blue assay color to yellow.

SPECIMEN COLLECTION AND PREPARATION

Coagulate blood and remove serum. Freeze sample at -20 °C or lower if not used immediately.

Do not heat inactivate serum.
Avoid repeated freezing and thawing of samples.

MATERIALS AND COMPONENTS

Materials provided with the test kits

1. **Plate:** Microwells containing *Schistosoma* SEA antigens - 96 test wells in a test strip holder.
2. **Enzyme Conjugate**: One (1) bottle containing 11 ml of Protein A Peroxidase (HRP) in a stabilizing buffer with Thimerosal.
3. **Positive Control**: One (1) vial containing 1 ml of diluted rabbit *Schistosoma*-positive sera in buffer with Thimerosal.
4. **Negative Control:** One (1) vial containing 1 ml of diluted *Schistosoma*-negative human sera in buffer with Thimerosal.
5. **TMB Substrate Solution:** One (1) bottle containing 11 ml of the TMB tetramethylbenzidine (TMB).
6. **Wash Concentrate 20X**: One (1) bottle containing 25 ml of concentrated buffer and surfactant with Thimerosal.
7. **Dilution Buffer:** Two (2) bottles containing 30 ml of buffered protein solution with Thimerosal
8. **Stop Solution**: One (1) bottle containing 11 ml of 1 M phosphoric acid.

Materials required but not provided

1. Pipettes
2. Squeeze bottle for washing strips
3. DI water
4. Tubes for serum dilutions
5. ELISA plate reader with a 450 nm and a 620-650 nm filter (optional if results are read visually.

Preparation

Wash Buffer - Remove cap and add contents of bottle to 475 ml DI water. Place diluted wash buffer into a squeeze bottle.
Note: Washings consist of filling to the top of each well, shaking out the contents and refilling.
Avoid generating bubbles in the wells during the washing steps.

Test samples: Make a 1:40 dilution of patients' sera using the dilution buffer.

ASSAY PROCEDURE

1. Break off number of wells needed (two for controls plus number of samples) and place in strip holder.
2. Add 100 µl of negative control to well #1, 100 µl of positive control to well #2, and 100 µl of the diluted (1:40) test samples to the remaining wells.
   Note: Negative and positive controls are supplied as prediluted. Do not dilute.
3. Incubate at room temperature (15 °C to 25 °C) for 10 minutes.
4. Shake out contents and wash 3 times with diluted wash buffer.*
5. Add 2 drops of enzyme conjugate to each well.
6. Incubate at room temperature for 10 minutes.
7. Shake out contents and wash 3 times with wash buffer.
8. Add 2 drops of Chromogen to every well.
9. Incubate at room temperature for 5 minutes.
10. Add 2 drops of stop solution.
11. Zero ELISA reader on air, read wells at 450 nm with a reference filter at 620-650 nm or read results visually.

* Washings consist of using the diluted wash buffer to fill to the top of each well, shaking out the contents and refilling the wells for a total of 3 times.

Avoid generating bubbles in the wells during the washing steps.

Controls must be included each time the kit is run.

Diagnostic Automation/ Cortez Diagnostics, Inc.
21250 Califa St, Suite 102 and 116, Woodland Hills, CA 91367 USA Phone: 818-591-3030, Fax : 818-591-8383
Email: onestep@rapidtest.com Website: www.rapidtest.com

***CAUTION! WHEN USING AN AUTOMATED OR SEMI-AUTOMATED WASHING SYSTEM THE FOLLOWING MUST BE FOLLOWED. FAILURE TO DO SO WILL RESULT IN INADEQUATE WASHING OF THE WELLS AND MAY LEAD TO FALSE POSITIVE RESULTS!**

Washing Procedure for Auto and Semi-Automated Washers

- **Perform five (5) washes per step instead of three**
- **Set machine to "soak" for one minute between each step**
- **After each set of washings, slap wells against an absorbent towel.**

RESULTS

Spectrophotometer:
Zero ELISA reader on air. Read all wells using a bichromatic reading with filters at 450 nm and 620-650 nm.
Positive - Absorbance reading greater or equal to 0.2 OD units.

Negative - Absorbance reading less than 0.2 OD units.

Interpretation of Results -Visual
A sample should be interpreted as positive if the degree of color development is obvious and significant.

Troubleshooting

Problem: Negative control has substantial color development.
***Correction*:** Inadequate washings. Rerun test with more vigorous washings.

QUALITY CONTROL

The use of a positive and negative control allows easy validation of kit stability. For a valid test, the positive control must be over 0.5 OD units and the negative control must be under 0.2 units. Should the values fall outside these ranges, the kit should not be used.

PERFORMANCE CHARACTERISTICS

		Reference Method *	
		+	-
Diagnostic Automation,Inc.	+	12	6
	-	0	34

Positive Agreement: 100% (12/12)
Negative Agreement: 85% (34/40)

*Reference Method refers to a commercially available ELISA.

LIMITATIONS OF PROCEDURE

Serological results should be used as an aid in diagnosis and should not be interpreted as diagnostic by themselves.

PRECAUTIONS

1. Do not use solutions if they precipitate or become cloudy.
2. Wash concentrate may show crystallization upon storage at 4 °C. Crystallization will disappear after diluting to working strength.
3. Do not use serum that may have supported microbial growth, or is cloudy due to high lipid content. Samples high in lipids should be clarified before use.
4. Do not add azides to the samples or any of the reagents.
5. Controls and some reagents contain Thimerosal as a preservative.
6. Treat all sera as if capable of being infectious.
7. The negative control has been tested and found negative for Hepatitis B surface antigen and for the antibody to HIV by required test methods. Since no test can offer complete assurance that infectious agents are not present, this product should be used under appropriate safety conditions that would be used for any potentially infectious agent.

STORAGE

1. Reagents, strips and bottled components should be stored at 2-8 °C
2. Squeeze bottle containing diluted wash buffer may be stored at room temperature.

REFERENCES

1. Carvalho EM, Lima AAM. Schistosomiasis (Bilharziasis). In: Goldman L, Schafer AI, eds. Cecil Medicine. 24th ed. Philadelphia, Pa: Saunders Elsevier; 2011:chap 363.
2. Maguire JH. Trematodes (schistosomes and other flukes). In: Mandell GL, Bennett JE, Dolin R, eds. Principles and Practice of Infectious Diseases. 7th ed. Philadelphia, Pa: Elsevier Churchill Livingstone; 2009:chap 289.

ISO 13485
ISO 9001

Diagnostic Automation/Cortez Diagnostics, Inc.
21250 Califa Street, Suite 102 and 116,
Woodland Hills, California 91367 USA

Date Adopted	**2016-10-05**
REF **8209-35**	**AccuDiag™- Schistosoma IgG ELISA**
EC REP	**CEpartner4U, Esdoornlaan 13, 3951DB Maarn. The Netherlands.** www.cepartner4u.eu

Revision Date: 2015-02