ab211773 Anti-Schistosoma mansoni IgM Human ELISA Kit

Instructions for use:

For the qualitative immunoenzymatic determination of IgM antibodies against Schistosoma mansoni in human serum or plasma (citrate or heparin).

This product is for research use only and is not intended for diagnostic use.

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1. BACKGROUND

Abcam's anti-Schistosoma mansoni IgM Human *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit (ab211773) is designed for the qualitative immunoenzymatic determination of IgM antibodies against Schistosoma mansoni in human serum or plasma (citrate or heparin).

Schistosomes belong to the class of distomas (trematodes). They rank among the most frequent pathogens. Estimations originate in more than 200 million affected people. The mature parasites are 6 – 22 mm long. The most important species are Schistosoma mansoni, S. japonicum and S. haematobium. Schistosoma mansoni is common in Africa, South America and Middle East.

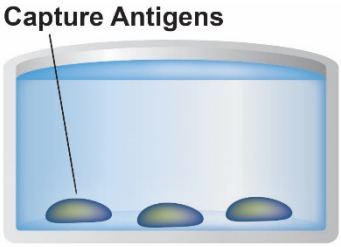
Schistosomiasis (bilharziosis) is, depending on species and location of the parasites, a disease of the intestine, liver and spleen resp. urinary passages. Humans are (re)infected by contact with fresh water which is contaminated by ova containing urine or faeces. If larvae bore into human skin, first a transient skin reaction appears (itch with exanthema or erythema, by repeatedly infection cercarial dermatitis is possible). After 3 to 10 weeks the now sexually mature worms synthesize cytotoxic and allergic substances which course feverish reaction in humans (Katayama fever). The infected person is mostly harmed by the eggs, which get into organs via blood excreting proteins and glycoproteins. The person reacts under participation of own antibodies and immune complexes with formation of granuloma and granulomatous proliferation in intestine and urinary bladder mucosa. Eggs not excreted die after 3 weeks and will be dissolved or calcified. The affected tissue gets fibrous. In final stage bilharziosis will cause death.

INTRODUCTION

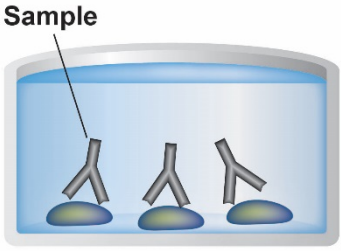
The qualitative immunoenzymatic determination of IgM antibodies against Schistosoma mansoni is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.

Microplates are pre-coated with Schistosoma mansoni antigens to bind corresponding antibodies of the sample. After washing the wells to remove all unbound sample material horseradish peroxidase (HRP) labelled anti-human IgM conjugate is added. This conjugate binds to the captured Schistosoma mansoni -specific antibodies. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product. The intensity of this product is proportional to the amount of Schistosoma mansoni -specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450 nm is read using an ELISA microwell plate reader.

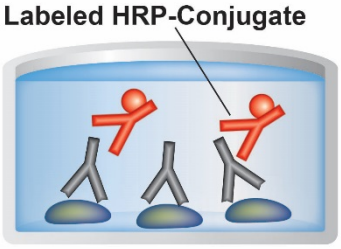
2. ASSAY SUMMARY



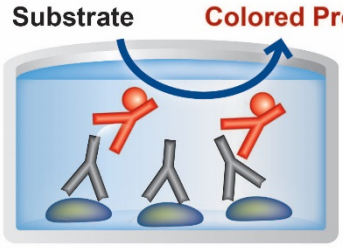
Prepare all reagents, samples, controls and standards as instructed.



Add samples and controls to wells used. Incubate at 37ºC.



Wash each well and add prepared labeled HRP-Conjugate. Incubate at room temperature.



After washing, add TMB substrate solution to each well. Incubate at room temperature. Add Stop Solution to each well. Read immediately.

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handle with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. STORAGE AND STABILITY

Store kit at 4°C immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

5. LIMITATIONS

- ELISA kit intended for research use only. Not for use in diagnostic procedures
- All components of Human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive. Nevertheless, all materials should still be regarded and handled as potentially infectious
- Use only clean pipette tips, dispensers, and lab ware
- Do not interchange screw caps of reagent vials to avoid cross-contamination
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination
- After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate, without splashing, accurately to the bottom of wells
- Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

6. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Schistosoma mansoni IgM Microplate	96 tests	4°C	4°C
IgM Sample Diluent	100 mL	4°C	4°C
Stop Solution	15 mL	4°C	4°C
20X Wash Buffer Concentrate	50 mL	4°C	4°C
Schistosoma mansoni anti-IgM Conjugate	20 mL	4°C	4°C
TMB Substrate Solution	15 mL	4°C	4°C
Schistosoma mansoni IgM Positive Control	2 mL	4°C	4°C
Schistosoma mansoni IgM Cut-off Control	3 mL	4°C	4°C
Schistosoma mansoni IgM Negative Control	2 mL	4°C	4°C
Cover foil	1 Unit	4°C	4°C

7. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully perform this assay:

- ELISA microwell plate reader, equipped for the measurement of absorbance at 450/620 nm
- Incubator 37 °C
- Manual or automatic equipment for rinsing wells
- Pipettes to deliver volumes between 10 and 1000 µL
- Vortex tube mixer
- Distilled water
- Disposable tubes

8. TECHNICAL HINTS

- Avoid foaming or bubbles when mixing or reconstituting components
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions
- Ensure plates are properly sealed or covered during incubation steps
- Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings
- Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction
- It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate
- The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions**

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.

9.1. Schistosoma mansoni IgM Microplate:

The ready to use break-apart snap-off strips are coated with Schistosoma mansoni antigens. Immediately after removal of the strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 4ºC.

9.2. IgM Sample Diluent:

1 bottle containing 100 mL of phosphate buffer (10 mM) for sample dilution; pH 7.2 ± 0.2; colored green; ready to use and stored at 4^0C.

9.3. Stop Solution:

1 bottle containing 15 mL sulphuric acid, 0.2 mol/L; ready to use and stored at 4^0C.

9.4. 20X Wash Buffer Concentrate:

1 bottle containing 50 mL of a 20-fold concentrated phosphate buffer (0.2 M); pH 7.2 ± 0.2; for washing the wells and stored at 4^0C. Dilute Washing Solution 1 + 19; e.g. 10 mL Washing Solution + 190 mL distilled water. The diluted buffer is stable for 5 days at room temperature

9.5. Schistosoma mansoni anti-IgM Conjugate

1 bottle containing 20 mL of peroxidase labelled antibody to human IgM; in phosphate buffer (10 mM); coloured blue, ready to use and stored at 4^0C.

9.6. TMB Substrate Solution

1 bottle containing 15 ml 3,3',5,5'-tetramethylbenzidine (TMB) < 0.1%; ready to use and stored at 4^0C away from the light. The solution should be colorless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.

9.7. **Schistosoma mansoni IgM Positive Control:**

1 bottle containing 2 mL control (human serum or plasma); colored yellow; ready to use and stored at 4^0C.

9.8. **Schistosoma mansoni IgM Cut-off Control:**

1 bottle containing 3 mL control (human serum or plasma); colored yellow; ready to use and stored at 4^0C.

9.9. **Schistosoma mansoni IgM Negative Control:**

1 bottle containing 2 mL control (human serum or plasma); colored yellow; ready to use and stored at 4^0C.

9.10. **Cover foil**

Ready to use and stored at 4^0C.

10. SAMPLE COLLECTION AND PREPARATION

- Use human serum or plasma (citrate or heparin) samples with this assay. If the assay is performed within 5 days after sample collection, the samples should be kept at 4°C; otherwise they should be aliquoted and stored deep-frozen (-70 to -20 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.
- Heat inactivation of samples is not recommended.
- Before assaying, all samples should be diluted 1+100 with IgM Sample Diluent. Dispense 10 µL sample and 1 mL Sample Diluent into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

11. ASSAY PROCEDURE

- **Please read the instruction for use carefully before performing the assay. Result reliability depends on strict adherence to the instruction for use as described.**
- **The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend increasing the washing steps from three to five and the volume of Washing Solution from 300 µL to 350 µL to avoid washing effects.**
- **Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established Select the required number of microtiter strips or wells and insert them into the holder.**
- **Perform all assay steps in the order given and without any delays.**
- **A clean, disposable tip should be used for dispensing each standard/control and sample.**
- **Adjust the incubator to 37 ± 1 °C.**

11.1. Dispense 100 µL standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.

11.2. Cover wells with the foil supplied in the kit.

11.3. Incubate for 1 hour ± 5 min at 37 ± 1 °C.

11.4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µL of Washing Solution. Avoid overflows from the reaction wells. The interval between washing und aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step.

Note: Washing is important! Insufficient washing results in poor precision and false results.

11.5. Dispense 100 µL Ascaris lumbricoides Protein A Conjugate into all wells except for the Substrate Blank well A1.

11.6. Incubate for 30 min at room temperature. Do not expose to direct sunlight.

11.7. Repeat step 11.4.

11.8. Dispense 100 µL TMB Substrate Solution into all wells.

11.9. Incubate for exactly 15 min at room temperature in the dark**.** A blue color occurs due to an enzymatic reaction.

11.10. Dispense 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate, thereby a color change from blue to yellow occurs.

11.11. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

12. CALCULATIONS

Adjust the ELISA Microwell Plate Reader to zero using the Substrate Blank.

If - due to technical reasons - the ELISA Microwell Plate Reader cannot be adjusted to zero using the Substrate Blank, subtract the absorbance value from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells at 450 nm and record the absorbance values for each standard/control and sample in the plate layout.

Bichromatic measurement using a reference wavelength of 620 nm is recommended.

Where applicable calculate the mean absorbance values of all duplicates.

In order for an assay to be considered valid, the following criteria must be met:

Run Validation Criteria

- **Substrate Blank**: Absorbance value **< 0.100**
- **Negative Control:** Absorbance value **< 0.200 and < Cut-off**
- **Cut-off Control:** Absorbance value **0.150 – 1.300**
- **Positive Control**: Absorbance value **> Cut-off**

If these criteria are not met, the test is not valid and must be repeated.

Calculation of Results

The Cut-off is the mean absorbance value of the Cut-off Control determinations.

Example:

Absorbance value Cut-off Control 0.44 + absorbance value Cut-off control 0.42 = 0.86 / 2 = 0.43

Cut-off = 0.43

Results in Abcam Units [AU]

Sample (mean) absorbance value x 10 = [Abcam Units = AU]

Cut-off

Example: 1.591 x 10 = 37 AU (Units)

0.43

Interpretation of Results

Cut- off	10 AU	
Positive	> 11 AU	Antibodies against the pathogen are present. There has been a contact with the antigen (pathogen resp. vaccine).
Equivocal	9 – 11 AU	Antibodies against the pathogen could not be detected clearly. It is recommended to repeat the test with a fresh sample in 2 to 4 weeks. If the result is equivocal again the sample is judged as negative.
Negative	< 9 AU	The sample contains no antibodies against the pathogen. A previous contact with the antigen (pathogen resp. vaccine) is unlikely.
Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. In immunocompromised patients and newborns serological data only have restricted value.		

13. TYPICAL SAMPLE VALUES

Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte. It is 92.6 %

PRECISION

	Intra-Assay	Inter-Assay
n=	24	21
CV (%)	8.23	3.8

Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte. It is 95.2 %

14. QUICK ASSAY PROCEDURE

***NOTE**: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.*

14.1. Diluted sample 1+100 with IgM Sample Diluent.

14.2. Dispense 100 µL standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.

14.3. Cover wells with the foil supplied in the kit.

14.4. Incubate for 1 hour ± 5 min at 37 ± 1 °C.

14.5. Aspirate and wash 3 times with 300 µL of Washing Solution. Tap strips on tissue paper prior to the next step.

14.6. Dispense 100 µL Ascaris lumbricoides Protein A Conjugate into all wells except for the Substrate Blank well A1.

14.7. Incubate for 30 min at room temperature. Do not expose to direct sunlight.

14.8. Repeat wash step.

14.9. Dispense 100 µL TMB Substrate Solution into all wells.

14.10. Incubate for 15 min at room temperature in the dark.

14.11. Dispense 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate.

14.12. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

15. TROUBLESHOOTING

Problem	Cause	Solution
Low signal	Incubation time to short	Repeat
	Precipitate can form in wells upon substrate addition when concentration of target is too high	Increase dilution factor of sample
	Using incompatible sample type	Detection may be reduced or absent in untested sample types
	Sample prepared incorrectly	Ensure proper sample preparation/dilution
Large CV	Bubbles in wells	Ensure no bubbles present prior to reading plate
	All wells not washed equally/thoroughly	Check that all ports of plate washer are unobstructed/wash wells as recommended
	Incomplete reagent mixing	Ensure all reagents/master mixes are mixed thoroughly
	Inconsistent pipetting	Use calibrated pipettes & ensure accurate pipetting
	Inconsistent sample preparation or storage	Ensure consistent sample preparation and optimal sample storage conditions (e.g. minimize freeze/thaws cycles)
High background	Wells are insufficiently washed	Wash wells as per protocol recommendations
	Contaminated wash buffer	Make fresh wash buffer
	Waiting too long to read plate after adding stop solution	Read plate immediately after adding stop solution
Low sensitivity	Improper storage of ELISA kit	Store all reagents as recommended. Please note all reagents may not have identical storage requirements.
	Using incompatible sample type (e.g. Serum vs. cell extract)	Detection may be reduced or absent in untested sample types

16. INTERFERENCES

These chemicals or biological materials will cause interference in this assay causing compromised results or complete failure:

- Interferences with hemolytic, lipemic or icteric samples are not observed up to a concentration of 10 mg/ml hemoglobin, 5 mg/ml triglycerides and 0.5 mg/ml bilirubin.

Cross Reactivity

- Cross reactions with antibodies against other pathogens, especially parasites and worms cannot be excluded.

17. NOTES

RESOURCES

RESOURCES

RESOURCES

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Email: technical@abcam.com | Tel: +44-(0)1223-696000

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