NOVATEINBIO
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Giardia Antigen in Stool ELISA Kit

Catalog number: NR-R10211 (96 wells)

The kit is designed to qualitatively detect *Giardia* Antigen in Stool

FOR **RESEARCH USE ONLY**. NOT FOR DIAGNOSTIC OR THERAPEUTIC PURPOSES

Important notes

- **The operation should be carried out in strict accordance with the provided instruction.**
- Store the unused strips in a sealed foil bag at 2-8°C.
- Always avoid foaming when mixing or reconstituting protein solutions.
- Pipette reagents and samples into the center of each well, avoid from bubbles.
- The samples should be transferred into the assay wells within 15 minutes of dilution.
- We recommend that the positive control, negative control and test samples are tested in duplicate.
- If the blue color develops too shallow after 10 minutes incubation with the substrates, it may be appropriate to extend the incubation time (Do not over develop).
- Avoid cross-contamination by changing tips, using separate reservoirs for each reagent.
- Avoid using the suction head without extensive wash.
- Do not mix the reagents from different batches.
- Stop Solution should be added in the same order of the Substrate solution.
- TMB reagent is light-sensitive. Avoid prolonged exposure to the light.

Intended use

This kit is used to qualitatively detect *Giardia* Antigen in Stool.

Assay time	90 min
Validity	Six months
Store at	2-8 °C

Assay principle

This assay employs the enzyme-linked immunosorbent assay (ELISA) technique to detect *Giardia* Antigen in the samples (Stool). Anti- *Giardia* antibodies are precoated in microplate wells. The *Giardia* Antigen in the samples, if presents, will bind to the antibody immobilized on the wells and interacts with HRP conjugated anti- *Giardia* antibodies to form an immunocomplex. Following incubation and washing procedures to remove unbound substances, this reaction is visualized by the addition of the chromogen tetramethylbenzidine (TMB). After stopping the reaction with sulfuric acid, the blue color turns yellow. What can be measured at this point is the amount of color intensity proportional to the amount of antigen captured in the wells, and to the sample.

Materials supplied

1	Microplate precoated with anti- Giardia antibodies	1x96 well
2	Negative Control	1 vial
3	Positive Control	1 vial
4	HRP anti- Giardia Ab conjugate	11ml
5	Wash Solution (20x)	25 ml
6	TMB developing reagent	11 ml
7	Stop Solution	11 ml
8	Closure plate membrane	2
9	Sealed bag	1
10	Package insert	1

Materials required but not supplied

- 37°C incubator.
- Stomacher or blender
- Standard microplate reader capable of measuring absorbance at 450 nm.

- Adjustable pipettes and disposable pipette tips.
- Distilled water.
- Multi-channel pipettes, manifold dispenser or automated microplate washer.
- Absorbent paper.
- Materials used for sample preparation.

Sample preparation and storage

Stool samples may be used as unpreserved or frozen, in Cary-Blair Transport Medium or in preservation media of 10% formalin or SAF. Mix well and briefly spin down to remove the particulate.

- Unpreserved samples should be kept at 2-8 °C and tested within 24 hours of collection. Samples that cannot be tested within this time should be frozen at -20 °C or lower until use. Avoid multiple freeze/thaw cycles. Prepare a 1:7 dilution of stool by adding 0.1 gram to 0.7 ml of diluted wash buffer before assay.
- Formalinized and SAF preserved samples may be kept at room temperature (22-25 °C) or at 2-8°C and tested within 18 months of collection. DO NO freeze preserved samples.
- Prepare a 1:1 dilution of stool by adding 1 gram to 4ml of diluted wash buffer before assay.

NOTE:

- Samples should be kept at 4°C and tested within 24 hours of collection. Samples that cannot be tested within this time should be frozen at -20 °C until use. When performing the assay, warm up samples to room temperature slowly. DO NOT USE HEAT-TREATED SAMPLES.
- Novateinbio is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient amount of samples in advance.
- If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
- Owing to the possibility of mismatching between antigen from other resource and antibody used in our kits (e.g., antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by our products.

- Influenced by the factors including cell viability, cell number and also sampling time, samples from cell culture supernatant may not be detected by the kit.
- Fresh samples are recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.

Reagent Preparation

- Bring all kit components and samples to room temperature (22-25 °C) before use.
- Wash Solution - Dilute 25 mL of Wash Solution concentrate (20×) with 475 mL of deionized or distilled water to prepare 500 mL of Wash Solution (1×).

Assay procedures

1. Prepare all the controls and samples before starting assay procedure (Please read Reagents Preparation).
2. Set one blank control well (no sample should be added into the Blank well), one Negative control well, one Positive control well, and test sample wells on the assay plate. Return unused strips to the resealable pouch with desiccant, seal, and store between 2° and 8°C.
3. Add 100 µl of Positive control, Negative control, and samples into their respective wells. Add 100 µl of Diluent buffer to the Blank well. Mix well. Cover and incubate the plate for 30 minutes at room temperature (22-25°C).
4. Wash the Microtiter Plate using one of the specified methods indicated below.
   Manual Washing: Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Fill in each well completely with diluted wash solution, and then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure four times for a total of FIVE washes. After washing, invert plate, and blot dry by hitting the plate onto absorbent paper or paper towels until no moisture appears. Note: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame. Complete removal of liquid at each step is essential to good performance.

   Automated Washing: Wash plate FIVE times with diluted wash solution (350-400 µl/well/wash) using an auto washer. After washing, dry the plate as above. It is recommended that the washer be set for a soaking time of 10 seconds and shaking time of 5 seconds between each wash.

5. Add 100 µl of HRP- Giardia Ab conjugate into each well, mix well. Cover and incubate the plate for 30 minutes at room temperature.
6. Discard the solution and wash the plate as step 4.
7. Add 100 µl of TMB developing reagent to each well, subsequently. Cover and incubate for 10-15 minutes at room temperature (Protect from light. Do not over develop).
8. Add 50 µl Stop Solution to each well, following the same order of TMB developing reagent addition. Mix well.
9. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader ***within 30 minutes after the addition of the Stop Solution.***

Determine the Results

Quality control

The use of controls allows validation of kit stability. If the following conditions are not met, the test should be considered invalid and should be repeated.

1. Negative control OD values: ≤0.15 (read against blank control)
2. Positive control OD values: ≥ 0.5 (read against blank control)

Interpretations of the results

1. Negative results: OD values ≤0.08 indicates no significant amounts of Giardia Antigens were detected.
2. Positive results: OD values >0.08 indicate that Giardia Antigens were detected.

Background

Giardia lamblia is the protozoan parasite responsible for the disease giardiasis. Symptoms of acute giardiasis include diarrhea, nausea, weight loss, malabsorption, abdominal cramps, flatulence and anemia. The disease may manifest itself as an acute, chronic or as an asymptomatic infection. The mode of transmission of *Giardia* is through fecal-oral ingestion of cysts.

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