Instructions for Use

Giardia Ag (stool) vet ELISA

RUO

REF EIA-6104

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Please use only the valid version of the Instructions for Use provided with the kit.
Verwenden Sie nur die jeweils gültige, im Testkit enthaltene, Gebrauchsanweisung.
Si prega di usare la versione valida delle istruzioni per l'uso a disposizione con il kit.
Por favor, se usa solo la version valida de la metodico técnico incluido aqui en el kit.
Utilisez seulement la version valide des Instructions d'utilisation fournies avec le kit.

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For Research Use Only

The test is intended for the detection of Giardia in diluted **veterinary stool specimen**.

1 PRINCIPLE OF THE ASSAY

The **qualtitative** immunoenzymatic determination of Giardia antigens is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.

Microtiter strip wells are precoated with Anti-Giardia antibodies to bind corresponding Giardia antigens of the specimen. After washing the wells to remove all unbound sample material horseradish peroxidase (HRP) labelled Anti-Giardia conjugate is added. This conjugate binds to the captured Giardia specific antigens. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) Substrate Solution which gives a blue reaction product. The intensity of this product is proportional to the amount of Giardia specific antigens in the specimen. 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 MATERIALS

2.1 Reagents supplied

- **Microtiter Plate:**
  12 breakapart 8-well snap-off strips coated with Anti-Giardia antibodies; in resealable aluminium foil.
- **Sample Dilution Buffer:**
  1 bottle containing 100 mL of phosphate buffer (10 mM) for sample dilution; pH 7.2 ± 0.2; coloured yellow; ready to use; white cap.
- **Stop Solution**:
  1 bottle containing 15 mL sulphuric acid, 0.2 mol/L; ready to use; red cap.
- **Washing Buffer (20x conc.):**
  1 bottle containing 50 mL of a 20-fold concentrated phosphate buffer (0.2 M), pH 7.2 ± 0.2, for washing the wells; white cap.
- **Conjugate:**
  1 bottle containing 15 mL of peroxidase labelled anti-Giardia antibodies; coloured red, ready to use; white cap.
- **TMB Substrate Solution:**
  1 bottle containing 15 mL 3,3',5,5'-tetramethylbenzidine (TMB), < 0.1 %; ready to use; yellow cap; < 5 % NMP (for hazard and precautionary statements see 12.1).
- **Positive Control:**
  1 bottle containing 2 mL; coloured yellow; ready to use; red cap.
- **Cut-off Control:**
  1 bottle containing 3 mL; coloured yellow; ready to use; green cap.
- **Negative Control:**
  1 bottle containing 2 mL; coloured yellow; ready to use; blue cap.

For potential hazardous substances please check the safety data sheet.

2.2 Materials supplied

- 1 Cover foil
- 1 Instruction for use (IFU)
- 1 Plate layout

2.3 Materials and Equipment needed

- 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 µL and 1000 µL
- Vortex tube mixer
- Distilled water
- Disposable tubes

2.4 STABILITY AND STORAGE

Store the kit at 2 °C - 8 °C.

The opened reagents are stable up to the expiry date stated on the label when stored at 2 °C - 8 °C.

2.5 REAGENT PREPARATION

It is very important to bring all reagents and samples to room temperature (20 °C - 25 °C) and mix them before starting the test run!

2.6 Coated Microplate

The break-apart snap-off strips are coated with Anti-Giardia antibodies.

Immediately after removal of the strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2 °C - 8 °C.

2.7 Washing Buffer (20x conc.)

Dilute Washing Buffer 1 + 19; e.g. 10 mL Washing Buffer + 190 mL distilled water.

The diluted buffer is stable for 5 days at room temperature (20 °C - 25 °C). In case crystals appear in the concentrate, warm up the solution to 37 °C e.g. in a water bath. Mix well before dilution.

2.8 TMB Substrate Solution

The reagent is ready to use and has to be stored at 2 °C - 8 °C, away from the light.

The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.

3 SAMPLE COLLECTION AND PREPARATION

The test is intended for the detection of Giardia in diluted **veterinary stool specimen**.

Either fresh or frozen specimen may be used in this test. If samples are stored frozen, thaw sample quickly, warm it to room temperature and mix thawed samples well before dilution.

If the assay is performed within 72 hours after sample collection, the specimen should be kept at 2 °C - 8 °C; otherwise they should be aliquoted and stored deep-frozen (-20 °C). Avoid repeated freezing and thawing.

3.1 Sample Dilution

Before assaying, all fresh or thawed samples should be diluted **1+ 9** with Sample Dilution Buffer.

Pipette 900 µL Sample Dilution Buffer into a clean tube.

Using a disposable stirring rod transfer about 100 mg (about 2-3 mm diameter) of faeces if solid or pipette 100 µL if liquid into the tube with Sample Dilution Buffer and suspend thoroughly, e.g. on a vortex.

Allow floating particles to sediment for 10 minutes at most. If a stool suspension sediments longer than 10 minutes the sample should be mixed again immediately before starting the assay.

4 ASSAY PROCEDURE

4.1 Test Preparation

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 Buffer from 300 µL to 350 µL to avoid washing effects. Pay attention to chapter 12. Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established on the plate layout supplied in the kit. 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 °C ± 1 °C.

1. Dispense 100 µL standards/controls and diluted samples into their respective wells.
   Leave well A1 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37 °C ± 1 °C.**
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 Buffer. Avoid overflows from the reaction wells. The interval between washing and 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.
5. Dispense 100 µL Conjugate into all wells except for the Substrate Blank well A1.
6. **Incubate for 30 min at room temperature (20 °C - 25 °C).** Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µL TMB Substrate Solution into all wells.
9. **Incubate for exactly 15 min at room temperature (20 °C - 25 °C) in the dark.**
   A blue colour occurs due to an enzymatic reaction.
10. Dispense 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution, thereby a colour change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

4.2 Measurement

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 its 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.

5 RESULTS

5.1 Run Validation Criteria

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

- **Substrate Blank**: Absorbance value **< 0.100**
- **Negative Control**: Absorbance value **< 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.

5.2 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

5.2.1 Results in Units [DU]

$$\frac{\text{Sample (mean) absorbance value} \times 10}{\text{Cut-off}} = \text{[DRG Units = DU]}$$

Example: $\frac{1.591 \times 10}{0.43} = 37$ DU

5.3 Interpretation of Results

Cut-off	10 DU
Positive	> 11 DU
Equivocal	9 – 11 DU
Negative	< 9 DU

6 LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

There is no correlation between the measured absorbance and the seriousness of the infection. It is also not allowed to correlate absorbance of the sample with that of the positive control. Cross-contamination of reagents and samples can produce false results. Incorrect dilutions, not sufficiently homogenized samples and samples, which stayed for sedimentation for more than 10 minutes can cause false results.

A negative ELISA result does not exclude a Giardia infection, because the excretion of cysts is periodic. Thus at least one further stool specimen of the regarding person should be demanded in case of a negative test result but clinical suspect.

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.

7 PRECAUTIONS AND WARNINGS

- **For research use only!**
- All materials of human or animal origin should be regarded and handled as potentially infectious.
- 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.
- Do not interchange reagents or strips of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- 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 standard/control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette samples and dispense reagents without splashing accurately into the wells.
- The ELISA is only designed for qualified personnel who are familiar with good laboratory practice.

7.1 Safety note for NMP-containing reagents

The TMB Substrate Solution contains NMP.

Therefore, the following hazard and precautionary statements apply.

Danger

H360D May damage the unborn child.

P280 Wear protective gloves, protective clothing, eye protection.

P308 + P313 IF exposed or concerned: Get medical advice/ attention.

Further information can be found in the safety data sheet.

7.2 Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

- **Abbreviations**

NMP	N-Methyl-2-pyrrolidone

8 SCHEME OF THE ASSAY

Test Preparation

Prepare reagents and samples as described.

Establish the distribution and identification plan for all samples and standards/controls on the plate layout supplied in the kit.

Select the required number of microtiter strips or wells and insert them into the holder.

Assay Procedure

	Substrate Blank (A1)	Negative Control	Cut-off Control	Positive Control	Sample (diluted 1+ 9)
Negative Control	-	100 µL	-	-	-
Cut-off Control	-	-	100 µL	-	-
Positive Control	-	-	-	100 µL	-
Sample (diluted 1+ 9)	-	-	-	-	100 µL
Cover wells with foil supplied in the kit **Incubate for 1 h at 37 °C** Wash each well three times with 300 µL of Washing Buffer					
Conjugate	-	100 µL	100 µL	100 µL	100 µL
Incubate for 30 min at room temperature (20 °C - 25 °C) Do not expose to direct sunlight Wash each well three times with 300 µL of Washing Buffer					
TMB Substrate solution	100 µL	100 µL	100 µL	100 µL	100 µL
Incubate for exactly 15 min at room temperature (20 °C - 25 °C) in the dark					
Stop Solution	100 µL	100 µL	100 µL	100 µL	100 µL
Photometric measurement at 450 nm (reference wavelength: 620 nm)					

SYMBOLS USED

Symbol	English	Deutsch	Italiano	Español	Français
	Consult instructions for use *	Gebrauchsanweisung beachten *	Consultare le istruzioni per l'uso	Consulte las instrucciones de uso	Consulter les instructions d'utilisation
REF	Catalogue number *	Artikelnummer *	Numero di Catalogo	Nûmero de catálogo	Référence de catalogue
LOT	Batch code *	Chargencode *	Codice del lotto	Codigo de lote	Numéro de lot
	Contains sufficient for <n> tests *	Ausreichend für <n> Prüfungen *	Contenuto sufficiente per "n" saggi	Contenido suficiente para <n> ensayos	Contenu suffisant pour "n" tests
	Temperature limit *	Temperaturbegrenzung *	Temperatura di conservazione	Temperatura de conservacion	Température de conservation
	Use-by date *	Verwendbar bis *	Utilizzare prima del	Establa hasta	Utiliser jusque
	Manufacturer *	Hersteller *	Fabbricante	Fabricante	Fabricant
	Caution *	Achtung *			
RUO	For research use only	Nur für Forschungszwecke	Solo a scopo di ricerca	Sólo para uso en investigación	Seulement dans le cadre de recherches
Distributed by	Distributed by	Vertreiber	Distributore	Distribuidor	Distributeur
Content	Content	Inhalt	Contenuto	Contenido	Contenu
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantità	Volumen/Número	Volume/Quantité
MTP	Microplate	Mikrotiterplatte	Micropiastra	Microplaca	Microplaque
CONJ	Conjugate	Konjugat	Coniugato	Conjugado	Conjugué
CONTROL –	Negative Control	Negativkontrolle	Controllo negativo	Control positivo	Contrôle négatif
CONTROL +	Positive Control	Positivkontrolle	Controllo positivo	Control positivo	Contrôle positif
CUT OFF	Cut off control	Cut off-Kontrolle	Controllo cut-off	Control cut-off	Contrôle cut-off
CAL	Standard or Calibrator	Standard oder Kalibrator	Standard o Calibratore	Estándar o Calibrador	Standard o Etalon
DIL	Sample Diluent	Probenverdünnungspuffer	Tampone diluente	Diluyente de la muestra	Diluant pour échantillon
SOLN STOP	Stop solution	Stopplösung	Soluzione bloccante	Solución de parada	Solution d'arrêt
SUB TMB	TMB Substrate solution	TMB-Substratlösung	Soluzione substrato TMB	Solución de sustrato de TMB	Solution de substrat TMB
WASH BUF 20x	Washing Buffer 20x concentrated	Waschpuffer 20x konzentriert	Tampone di lavaggio concentrazione x20	Tampón de lavado concentrado x20	Tampon de lavage concentré 20 x