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ProSpecT™ Giardia Microplate Assay

REF R2458024 .....24 Tests
REF R2458096 .....96 Tests

1 INTENDED USE

ProSpecT Giardia Microplate Assay uses monoclonal antibody for the qualitative detection of *Giardia* Specific Antigen (GSA 65) in aqueous extracts of faecal specimens.

2 SUMMARY

Giardiasis is now recognized as an important human intestinal disease in most areas of the world. The causative organism, *Giardia lamblia*, is the most frequently identified protozoan parasite in stool specimens submitted to U.S. public health laboratories[3]. This parasite has been implicated in a number of epidemics[4,5] and the endemicity in the U.S. is well-recognized. Prevalence in adults is estimated at 4-7%[8]. Higher prevalence rates have been reported in children[1,15] and in homosexual males[6,7]. Acute symptoms of giardiasis may include diarrhoea, malabsorption, abdominal cramps, anorexia, nausea, weight loss, flatulence, anaemia, and general weakness lasting from several weeks to several months[16]. Chronic infections can also occur with or without an acute phase, are often associated with treatment failure, and may result in recurrent symptoms. Infection with *Giardia* may also be asymptomatic[2].

Giardia Specific Antigen (GSA 65) is a macromolecule that has been found in association with *Giardia* infections and has been used as the basis of immunoassays[9,10,11,12]. GSA 65 is a 65,000 molecular weight glycoprotein that is produced in abundant quantities by the *Giardia lamblia* protozoa as they multiply within the host intestinal tract. The antigen is present only when *Giardia* infection is present and it is possible to find GSA 65 in stool specimens without visible signs of cysts or trophozoites[9,10,11]. GSA 65 is a *Giardia* Specific Antigen and anti-GSA 65 antibodies have not been found to cross react with other enteric parasites[1,10]. GSA 65 is stable to transport through the host intestinal tract as well as to most routine procedures used to collect and transport stool specimens for ova and parasite (O&P) microscopic examination[1,11,12,13].

3 PRINCIPLE OF THE TEST

ProSpecT Giardia Microplate Assay is a solid phase immunoassay for the detection of GSA 65[14]. Diluted stool specimens are added to break-away microplate wells on which anti-GSA 65 antibody is bound. If GSA 65 is present, it is 'captured' by the bound antibody. The wells are incubated and then washed to remove unbound material. The enzyme conjugate (monoclonal anti-GSA antibody labelled with horseradish peroxidase enzyme) is added. The wells are incubated and then washed to remove unbound enzyme conjugate. In a positive reaction, GSA 65 binds the enzyme conjugate to the well. The substrate for the enzyme, 3,3',5,5'-tetramethylbenzidine (TMB), is added. In a positive reaction, the enzyme bound to the well by GSA 65 converts the substrate to a coloured reaction product. Colour development can be detected visually or spectrophotometrically. In a negative reaction, there is no GSA 65 or an insufficient level of GSA 65 present to bind the enzyme conjugate and no coloured reaction product develops.

4 SYMBOL DEFINITIONS

REF	Catalogue Number
IVD	In Vitro Diagnostic Medical Device
Σ	Contains sufficient for <n> tests
	Consult Instructions for Use (IFU)
8°C 2°C	Temperature Limitation (Storage Temp.)
LOT	Batch Code (Lot Number)
	Use By (Expiration Date)
	Manufacturer
DILUTED SAMPLE	Diluted Sample

5 KIT CONTENTS, PREPARATION FOR USE AND STORAGE

The ProSpecT Giardia Microplate Assay includes sufficient reagents to perform Σ 24 or Σ 96 tests.
See also **Precautions,** section **6**.

The expiration date of each kit is stated on the package label.

Store all components at 2 to 8°C.

Before use, bring all reagents to room temperature (20 - 25°C) and mix gently. Return the unused reagents to the refrigerator after use.

All reagents, except the Wash Buffer, are supplied at working strength. Reagents can be dispensed directly from the dropper bottles or poured out for use with multichannel pipettes. If excess reagent has been poured, the excess should be discarded. Do not pour excess reagent back into the bottle.

Instructions for Use
Transfer pipettes
Microplate Strip Holder and Cover
Procedure Card

MICROTITRATION PLATE
Microplate* (8 wells / strip)
3 strips (R2458024) or 12 strips (R2458096) coated with rabbit anti-GSA 65 antibody. Unused microplate strips should be stored in the foil pouch containing desiccant to exclude moisture.

CONJUGATE
Enzyme Conjugate*
One dropper bottle containing 5 ml (R2458024) or 25 ml (R2458096) of horseradish peroxidase labelled mouse monoclonal anti-GSA with bovine serum and antimicrobial agents.

CONTROL +
Positive Control
One dropper bottle containing 4 ml of a buffered solution with inactivated Giardia antigen, and antimicrobial agents.

CONTROL –
Negative Control
One dropper bottle containing 4 ml of a buffered solution with a red dye, and antimicrobial agents.

SAMPLE DILUENT
Specimen Dilution Buffer
One bottle containing 35 ml (R2458024) or 120 ml (R2458096) of a buffered solution with rabbit serum, a red dye and antimicrobial agents.

WASH BUFFER (x10)
Wash Buffer
One bottle containing 50 ml (R2458024) or 120 ml (R2458096) of a (x10) concentrated buffered solution with antimicrobial agents.
Dilute (x10) Wash Buffer concentrate to (x1) by adding 1 part concentrate to 9 parts distilled or deionised water. Diluted Wash Buffer is stable for 1 month when stored at 2 - 8°C.

SUBSTRATE TMB
Colour Substrate
One dropper bottle containing 12 ml (R2458024) or 25 ml (R2458096) of 3,3',5,5'-tetramethylbenzidine (TMB) in buffer.
The Colour Substrate should be stored in and used from the light protected bottle in which it is provided. If an aliquot is removed from the original bottle for any reason, do not return unused Colour Substrate to the original bottle.

STOP SOLUTION
Stop Solution
One dropper bottle containing 12 ml of 0.46 mol/l Sulphuric acid.

***Note:** Do not interchange reagents between kits with different lot numbers.

6 PRECAUTIONS

IVD

The reagents are for *in vitro* diagnostic use only.

For professional use only.

Please refer to the Material Safety Data Sheet (MSDS) and product labelling for information on potentially hazardous components.

HEALTH AND SAFETY INFORMATION

6.1 Reagents are prepared from biological materials and should be handled as potentially infectious material. Discard using appropriate biohazard procedures.

6.2 Do not pipette by mouth. Wear disposable gloves and eye protection while handling specimens and performing the assay. Wash hands thoroughly when finished.

6.3 Specimens may contain potentially infectious agents and should be handled at Biosafety Level 2 as recommended in the CDC/NIH manual, "Biosafety in Microbiological and Biomedical Laboratories", 5th Edition.

6.4 Wash Buffer contains potential skin sensitiser (< 1% v/v). Avoid skin contact. Wear disposable Vinyl or Nitrile gloves.

6.5 Discard used Wash Buffer in appropriate biohazard containers.

ANALYTICAL PRECAUTIONS

6.6 Carefully read and follow all instructions in this Instruction for Use.

6.7 Reagents are provided at the necessary working strength, with the exception of the Wash Buffer concentrate. Do not dilute reagents, except where instructed.

6.8 Do not use reagents beyond the expiration dates. Expiration dates are printed on each reagent label. Use of reagents beyond the expiration date may affect the accuracy of results.

6.9 The following common reagents may be used across the ProSpecT product range: Wash Buffer, Colour Substrate and Stop Solution.

6.10 Microbial contamination of reagents may decrease the accuracy of the assay. Avoid microbial contamination of reagents by using sterile disposable pipettes when removing aliquots from reagent bottles.

6.11 Allow all reagents and specimens to reach room temperature (20 - 25°C) before use.

6.12 Microplate strips must be stored in the resealable foil pouch, with desiccant, to protect microplate wells from moisture.

6.13 Stool samples must be thoroughly mixed prior to specimen processing to ensure accurate representation of the specimen. DO NOT CONCENTRATE SPECIMENS BEFORE TESTING.

6.14 Colour Substrate is sensitive to light exposure. If the reagent is exposed to light and develops colour, the reagent must be discarded.

6.15 Persons who are colour blind or visually impaired may not be able to read the test visually and should use spectrophotometric readings to interpret results.

6.16 Add reagents to the test wells in the same order throughout the procedure. To avoid contamination do not touch the fluid in the wells with the bottle tips.

6.17 Time each incubation accurately. Start timing after adding reagent to the last well on each microplate being tested. To ensure accurate timing, process no more than three 96 well plates at one time. Deviation from the established procedure may alter the performance of the assay.

6.18 It is important to hold the dropper bottles vertically and that the drop forms at the tip of the nozzle. If the nozzle becomes wet, a drop of incorrect volume will form around the end and not at the tip; if this occurs dry the nozzle before progressing.

7 COLLECTION OF FAECAL SPECIMENS

Specimens collected for routine ova and parasite examination can be used for the ProSpecT Giardia Microplate Assay. Stool specimens should be collected in clean, leak-proof plastic containers.

FRESH Untreated stool specimens should be stored at 2 - 8°C and tested within 48 hours.

FROZEN If fresh specimens cannot be tested within 48 hours, they should be frozen at -20 to -70°C.

PRESERVED Stool specimens treated with 10% formalin, MF or SAF fixatives may be refrigerated (2 - 8°C) or stored at room temperature (20 - 25°C) and should be tested within 2 months after collection.

CARY BLAIR Stool specimens collected in Cary Blair Transport Medium (or equivalent) should be refrigerated or frozen and tested within 1 week after collection. Stool specimens that have been concentrated or treated with PVA fixatives are not suitable for use.

SWAB/DIAPER Stool specimens obtained from rectal swabs and diapers are acceptable for use in the ProSpecT Giardia Microplate Assay. Please note the use of super absorbent diapers is not acceptable.

8 TEST PROCEDURE

REQUIRED MATERIALS PROVIDED

See **Kit Contents**, section **5**

MATERIALS REQUIRED BUT NOT PROVIDED

Stool specimen collection containers
Timer that measures minutes
Wash bottle for Wash Buffer
Distilled or deionised water

OPTIONAL MATERIALS NOT PROVIDED

Microplate reader capable of reading 450 nm or 450/620 to 650 nm
Cotton or rayon tipped applicator sticks
Micropipette to deliver volumes to 200 µl
Plastic or glass disposable test tubes
Vortex mixer with plate adapter or shaker

PROCEDURE

8.1 Open the foil pouch, remove the required number of microplate strips and place into a microplate strip holder. Use one well for the Negative Control and one well for the Positive Control. If using less than 8 wells, break off the required number of wells from a strip and return the unused wells to the foil pouch with desiccant. RESEAL POUCH TIGHTLY TO EXCLUDE MOISTURE AND RETURN TO THE REFRIGERATOR.

8.2 Specimens can be added directly into the wells or pre-diluted in tubes before adding to the wells. Pre-diluted specimens can be held at room temperature (20 - 25°C) for 8 hours or at 2 - 8°C for 48 hours prior to testing (see below). Choose one of these two methods: See Box "**A**" for dilution in wells; See Box "**B**" for dilution in tubes.

	A Dilutions in Wells
1	**Unpreserved Solid Specimens:** Label one tube for each specimen. Add **0.4 ml** Specimen Dilution Buffer (SDB) to each tube. Coat **1 swab** with specimen and vigorously mix into SDB. Express as much fluid as possible and discard the swab. Put a transfer pipette into the tube.
2	**Preserved or Watery Unpreserved Specimens:** Mix by shaking specimen collection containers. No further preparation is necessary.
3	Add **4 drops** Negative Control to well A1. Add **4 drops** Positive Control to well B1.
4	Add **100 µl** SDB to each specimen well.
5	Using transfer pipettes add **1 drop** of each specimen to a well. Note: Place the opening of the transfer pipettes just inside the wells to avoid splashing into adjacent wells.
6	**PROCEED TO STEP 8.3**

	B Dilution in Tubes
1	**Unpreserved Solid Specimens:** Label one tube for each specimen. Add **1 ml** Specimen Dilution Buffer (SDB) to each tube. Coat **1 swab** with specimen and vigorously stir into SDB. Express as much fluid as possible and discard the swab. Put a transfer pipette into each tube.
2	**Preserved or Watery Unpreserved Specimens:** Label one tube for each specimen. Add **1 ml** SDB to each tube. Mix samples by shaking specimen collection containers. Using transfer pipettes draw up **0.3 ml** (third mark from the tip of the pipette). Expel sample into SDB. Mix by drawing up and down once. Leave transfer pipettes in the tubes. **Diluted specimens may be held for 8 hours at room temperature (20 - 25°C) or 48 hours at 2 - 8°C.**
3	Add **4 drops** Negative Control to well A1.
4	Add **4 drops** Positive Control to well B1.
5	Using transfer pipettes add **0.2 ml** (second mark from the tip of the pipette) of each specimen to a well. Note: Place the opening of the transfer pipettes just inside the wells to avoid splashing into adjacent wells.
6	**PROCEED TO STEP 8.3**

8.3 **Cover** microplate and incubate at room temperature (20 - 25°C) for **60 minutes**. Begin timing after the addition of the last specimen.

8.4 Shake out or aspirate the contents of the wells. Wash by completely filling each well with **diluted** Wash Buffer (~350- 400 µl/well). Shake out or aspirate all fluid from the wells after each wash. Wash a total of **3 times**. After the last wash remove contents and strike plate on clean paper towels or aspirate. Remove as much Wash Buffer as possible but do not allow the wells to dry out at any time.

8.5 Add **4 drops** (200 µl) of Enzyme Conjugate to each well.

8.6 **Cover** microplate and incubate at room temperature (20 - 25°C) for **30 minutes.**

8.7 Shake out or aspirate and wash each well **5 times** as in step 8.4.

8.8 Add **4 drops** (200 µl) of Colour Substrate to each well.

8.9 **Cover** microplate and incubate at room temperature (20 - 25°C) for **10 minutes.**

8.10 Add **1 drop** (50 µl) Stop Solution to each well. Gently tap or vortex the wells until the yellow colour is uniform. Read reactions within **10 minutes** after adding the Stop Solution.

8.11 Read visually or spectrophotometrically at 450 nm (single wavelength) or 450/620 to 650 nm (dual wavelength).

9 QUALITY CONTROL

Positive and Negative Controls must be included each time the test is performed. The Positive and Negative Controls serve as both reagent and procedural controls. The controls are intended to monitor for substantial reagent failure. The Positive Control will not ensure precision at the assay cut-off.

The optical density (O.D.) of the Negative Control should be ≤ 0.100 at 450 nm or < 0.070 at 450/620 to 650 nm. The Negative Control should be colourless when read visually. If yellow colour equal to 1+ or greater on the Procedure Card is present in the Negative Control, the test should be repeated with careful attention to the wash procedure.

The O.D. of the Positive Control should be ≥ 0.300 at 450 nm or 450/620 to 650 nm, after the O.D. of the Negative Control is subtracted and should be equal to or greater than the 2+ reaction when read visually. If yellow colour less than 2+ on the Procedure Card is present in the Positive Control, call for technical assistance.

10 RESULTS

Refer to the enclosed Procedure Card for colour interpretations.

VISUAL

10.1 Read the test results by comparing with the reaction colours on the Procedure Card.
Positive: yellow colour of at least 1+ intensity
Negative: colourless

10.2 Interpretation of visual results:
Positive: If yellow colour of at least 1+ intensity develops in the test well, the sample contains GSA 65 and the test is positive.
Note: Tests with faint yellow colour (less than 1+) should be repeated.
Negative: A colourless reaction is a negative result and indicates that no GSA 65 or an undetectable level of GSA 65 is present in the sample tested.

SPECTROPHOTOMETRIC

10.3 Read results at either single (450 nm) or dual (450/620 to 650 nm) wavelength.

10.4 Read the optical density (O.D.) for the Negative Control.

10.5 Subtract the O.D. of the Negative Control well from the O.D. readings of the Positive Control well and the test wells before interpreting results.
Note: Readers may be set to blank on the Negative Control well so that the Negative Control well O.D. is automatically subtracted from all of the other readings. If the reader does not have this capability, blank on air and subtract the O.D. of the Negative Control well from the O.D. readings of the Positive Control well and test wells before interpreting results.

10.6 Read the test results:
Positive: O.D. of ≥ 0.050 blanked value
(i.e. after the O.D. of the Negative Control is subtracted)
Negative: O.D. of < 0.050 blanked value
(i.e. after the O.D. of the Negative Control is subtracted)

10.7 Interpretation of spectrophotometric results:
Positive: If the blanked O.D. reading is equal to or greater than 0.050 in the test well, the sample contains GSA 65 and the test is positive.
Negative: A blanked O.D. reading less than 0.050 is a negative result and indicates that no GSA 65 or an undetectable level of GSA 65 is present in the sample tested.
***Note:** Any wells that are visually clear but give an O.D. reading that is inconsistent with the visual interpretation should be considered a discrepant reading and examined for the presence of bubbles, small particles in the wells, or an opaque film on the bottom of the well. To remove the film, wipe the underside of the wells and read the O.D. again. If the discrepancy between visual and O.D. readings persists, repeat the test.

11 PERFORMANCE LIMITATIONS

The validity of results with the ProSpecT Giardia Microplate Assay depends on the control reaction performing as expected. See **Quality Control** section **9**.

A negative test result does not exclude the possibility of the presence of *Giardia*, and may occur when the antigen level in the sample is below the detection level of the test. Correlation between the amount of antigen in a sample and clinical presentation has not been established.

As with all IN VITRO diagnostic tests, results should be interpreted by the clinician in conjunction with clinical findings and/or other laboratory results.

Proper specimen collection and processing are essential to achieve optimal performance of the assay. Optimal test results are obtained from specimens tested as soon after collection as possible. See **Collection of Faecal Specimens** section **7**.

ProSpecT Giardia Microplate Assay has been classified as high complexity.

12 EXPECTED VALUES

The prevalence of *Giardia* infection varies in different populations and geographic areas. In the U.S., the incidence of *Giardia* is approximately 4-7% with higher prevalence rates in children[12] and in homosexual males[5,6].

13 PERFORMANCE CHARACTERISTICS

SENSITIVITY AND SPECIFICITY

Clinical studies were conducted to evaluate the performance of the ProSpecT Giardia Microplate Assay. Specimens were obtained from a large reference laboratory which performed O&P testing. A total of 248 unpreserved specimens were tested; 101 were positive for *Giardia* by O&P and 147 were negative. Forty-seven of the *Giardia* negative specimens contained parasites other than *Giardia* by O&P. All of the O&P positive specimens were positive in the microplate assay and all of the negative specimens were negative. The performance of the ProSpecT Giardia Microplate Assay in this study is presented below:

		O & P +	O & P –	
ProSpecT	+	101	0	
Giardia	–	0	147	
		101	147	248

Sensitivity 101/101 = 100% (96 - 100%)
Specificity 147/147 = 100% (98 - 100%)
Numbers in parentheses are 95% confidence intervals.

A trial was conducted with 562 prospectively collected specimens tested by O&P. The specimens were collected from a large metropolitan hospital reference laboratory (360 unpreserved specimens) and from a public health laboratory (202 specimens preserved in formalin). There was one O&P positive/EIA negative result and 10 specimens were O&P negative/EIA positive. One of these was GSA 65 positive by specific inhibition. The performance of the ProSpecT Giardia Microplate Assay in this trial is given in the following chart:

		O&P/Specific Inhibition		
		+	–	
ProSpecT	+	42	9	
Giardia	–	1	510	
		43	519	562

Sensitivity 42/43 = 98% (88 - 100%)
Specificity 510/519 = 98% (97 - 99%)
Numbers in parentheses are 95% confidence intervals.

ANALYTICAL SENSITIVITY

The ProSpecT Giardia Microplate Assay detects approximately 3.9 nanograms/ml of GSA 65.

REPRODUCIBILITY

The inter-assay or run-to-run coefficient of variation (CV) was evaluated with 5 positive and 5 negative samples assayed at least ten times in three separate runs. For the dilution in tube procedure the mean CV of the negative samples was 3.02% (range 0.93% to 3.90%) and the mean CV for the positive samples was 4.65% (range 1.74% to 10.72%). For the dilution in wells procedure the mean CV of the negative samples was 7.66% (range 4.22% to 16.19%) and the mean CV for the positive samples was 7.76% (range 4.12% to 11.26%).

The intra-assay or within-run CV was evaluated with 5 positive and 5 negative samples assayed at least ten times in a single run. For the dilution in tube procedure the mean CV of the negative samples was 4.47% (range 3.30% to 5.39%) and the mean CV for the positive samples was 5.61% (range 2.53% to 10.12%). For the dilution in wells procedure the mean CV of the negative samples was 4.51% (range 2.73% to 5.36%) and the mean CV for the positive samples was 9.58% (range 4.78% to 13.8%).

CROSS-REACTIVITY

The ProSpecT Giardia Microplate Assay has been tested with stool specimens found to be positive for a number of faecal organisms. No cross-reactivity was observed with any of the infectious agents listed below.

Ascaris lumbricoides (5)	*Entamoeba histolytica* (5)
Blastocystis hominis (6)	*Hymenolepis nana* (2)
Cryptosporidium parvum (10)	*Iodamoeba butschlii* (9)
Dientamoeba fragilis (10)	*Isospora belli* (5)
Endolimax nana (6)	Rotavirus (11)
Entamoeba coli (13)	*Strongyloides stercoralis* (1)
Entamoeba hartmanni (5)	*Trichuris trichiuria* (2)

Numbers in parentheses indicate the numbers of specimens tested.

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