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ProSpecT Cryptosporidium Microplate Assay EN

REF R2454024 .....24 Tests
REF R2454096 .....96 Tests

1 INTENDED USE

ProSpecT™ Cryptosporidium Microplate Assay uses monoclonal antibodies for the qualitative detection of *Cryptosporidium* Specific Antigen (CSA) in aqueous extracts of faecal specimens.

2 SUMMARY

Cryptosporidiosis has recently been recognized as an important human disease in most areas of the world[6,9]. The causative agent, *Cryptosporidium* spp., has been identified in stool specimens of children and adults in many countries and most states in the United States[6]. This parasite has been implicated in severe disease in HIV-infected persons[10], day-care centres[1,2,11,12] and waterborne outbreaks[5,7,8] in the United States. Groups at particular risk include immunocompromised persons, especially those with HIV infection, family members and sexual partners of infected patients, children and caretakers in child day-care centres, animal handlers and travellers[6]. Acute symptoms of cryptosporidiosis may include diarrhoea, abdominal pain, nausea and vomiting, fever, malaise, and respiratory problems lasting from several days to more than a month and often leading to persistent infection or death in immunologically deficient patients[6]. Infection with *Cryptosporidium* may also be asymptomatic.

Cryptosporidium specific antigens have been found associated with *Cryptosporidium* infections and have been used as the basis of fluorescent and antigen capture immunoassays[3,4,13]. The *Cryptosporidium* specific antigen (CSA) detected by this kit is produced by *Cryptosporidium* organisms as they multiply within the host intestinal tract. The antigen is specific to *Cryptosporidium* and has not been found to cross-react with other enteric parasites. The antigen is stable to transport through the host intestinal tract as well as to routine procedures used to collect and transport specimens for microscopic examination.

3 PRINCIPLE OF THE TEST

ProSpecT Cryptosporidium Microplate Assay is a solid phase immunoassay for the detection of CSA. Diluted stool specimens are added to break-away microplate wells on which anti-CSA antibody is bound. If CSA 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-CSA antibody labelled with horseradish peroxidase enzyme) is added. The wells are incubated and then washed to remove unbound enzyme conjugate. In a positive reaction, CSA 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 CSA converts the substrate to a coloured reaction product. Colour development can be detected visually or spectrophotometrically. In a negative reaction, there is no CSA or an insufficient level of CSA 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)
2°C 8°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 Cryptosporidium 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 (R2454024) or 12 strips (R2454096) coated with rabbit anti-CSA antibodies. Unused microplate strips should be stored in the foil pouch containing desiccant to exclude moisture.

CONJUGATE

Enzyme Conjugate*
One dropper bottle containing 5 ml (R2454024) or 25 ml (R2454096) of horseradish peroxidase labelled mouse monoclonal anti-CSA with bovine serum and antimicrobial agents.

CONTROL +

Positive Control
One dropper bottle containing 4 ml of *Cryptosporidium* Oocyst extract in a buffered solution with 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 (R2454024) or 120 ml (R2454096) of a buffered solution with rabbit serum, a red dye and antimicrobial agents.

WASH BUFFER (x10)

Wash Buffer
One bottle containing 50 ml (R2454024) or 120 ml (R2454096) 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 (R2454024) or 25 ml (R2454096) 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 Cryptosporidium 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 Cryptosporidium 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	**Dilution 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 **2 drops** 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 CSA 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 CSA or an undetectable level of CSA 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 CSA and the test is positive.
Negative: A blanked O.D. reading less than 0.050 is a negative result and indicates that no CSA or an undetectable level of CSA 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 LIMITATIONS OF THE PROCEDURE

The validity of results with the ProSpecT Cryptosporidium 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 *Cryptosporidium*, 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 Cryptosporidium Microplate Assay has been classified as high complexity.

12 EXPECTED VALUES

The prevalence of *Cryptosporidium* infection varies in different populations and geographic areas. In the U.S., the incidence of *Cryptosporidium* is approximately 0.5 - 3.0% 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 Cryptosporidium Microplate Assay with specimens obtained from large reference and hospital laboratories which performed O&P testing. A total of 214 specimens were tested; 81 were positive for *Cryptosporidium* by acid-fast (AF) and 133 were negative. Forty of the *Cryptosporidium* negative specimens contained organisms other than *Cryptosporidium* by AF or O&P.

The results of these evaluations are presented below:

		Acid Fast	
		+	–
ProSpecT	+	81	0
Cryptosporidium	–	0	133
		81	133 214

Sensitivity 81/81 = 100% (95.5 - 100%)
Specificity 133/133 = 100% (97.3 - 100%)
Numbers in parenthesis are 95% confidence intervals.

Clinical studies were conducted to evaluate the performance of the ProSpecT Cryptosporidium Microplate Assay. Specimens were obtained from hospital labs and the CDC. Patient populations represented in the specimen pool were symptomatic patients in normal prevalence populations, symptomatic patients in a high prevalence (HIV positive) population and asymptomatic patients from a day care population. Specimens were submitted unpreserved or preserved in 10% formalin or SAF. Samples were tested for *Cryptosporidium* by either acid-fast (AF) or immunofluorescent staining methods (IFA). A total of 212 specimens were tested; 134 were positive for *Cryptosporidium* specific antigen (CSA) and 78 were negative. The results with the ProSpecT Cryptosporidium Microplate Assay are presented below:

		Acid Fast	
		+	–
ProSpecT	+	130	0
Cryptosporidium	–	4	78
		134	78 212

Sensitivity 130/134 = 97% (92.5 - 99.2%)
Specificity 78/78 = 100% (95.4 - 100%)

Numbers in parenthesis are 95% confidence intervals.

A prospective trial was conducted at a large metropolitan hospital. All samples submitted for acid-fast staining for *Cryptosporidium* over a period of 4 months were included in the study. Samples were unpreserved and frozen at -20°C prior to testing with the ProSpecT Cryptosporidium Microplate Assay. The results of initial testing and the resolved data are presented below. Data was resolved by repeat testing of the 14 AF negative/ CSA positive samples. Six of the fourteen were reproducibly positive for CSA. Specific inhibition studies with antibody to CSA showed greater than 50% inhibition in all 6 samples. These 6 samples are considered to be true positives in the resolved data.

		Acid Fast		Resolved		
		+	–	+	–	
ProSpecT	+	28	14	34	8	
Cryptosporidium	-	1	335	1	335	
		29	349	35	343	378

Sensitivity 28/29 = 97% (82.2 - 99.9%) 34/35 = 97% (85.1 - 99.9%)
Specificity 335/349 = 96% (93.4 - 97.8%) 335/343 = 98% (95.5% - 99.0%)
Numbers in parentheses are 95% confidence intervals.

ANALYTICAL SENSITIVITY

The ProSpecT Cryptosporidium Microplate Assay detects approximately 20 nanograms/ml of CSA.

REPRODUCIBILITY

The inter-assay or run-to-run coefficient of variation (CV) of the ProSpecT Cryptosporidium Microplate Assay was evaluated by selecting 10 positive specimens with varying optical density readings. Each sample was tested in 10 wells per day for five days. The mean inter-assay CV was 10.6%.

The intra-assay or within-run CV was evaluated by testing 24 wells with each of 5 positive specimens. The mean intra-assay CV was 2.52%.

CROSS-REACTIVITY

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

Ascaris lumbricoides (2)
Blastocystis hominis (4)
Chilomastix mesnili (1)
Dientamoeba fragilis (4)
Endolimax nana (3)
Entamoeba coli (6)
Entamoeba hartmanni (2)
Entamoeba histolytica (5)
Giardia lamblia (5)
Hymenolepis nana (2)
Iodamoeba butschlii (2)
Isospora belli (2)
Strongyloides stercoralis (2)
Taenia solium (1)
Trichuris trichiura (1)

The numbers in parentheses indicate the number of specimens tested.

14 BIBLIOGRAPHY

1. **Alpert, G. et al., 1986.**
Outbreak of Cryptosporidiosis in a Day-Care Centre.
Pediatrics 77(2):152-157.

2. **Anon. 1984.**
Cryptosporidiosis among children attending day-care centres - Georgia, Pennsylvania, Michigan, California, New Mexico.
Morbid. Mortal. Weekly Rep. 33(42):599-601.

3. **Arrowood, M.J. and C.R. Sterling, 1989.**
Comparison of Conventional Staining Methods and Monoclonal Antibody-based Methods for *Cryptosporidium* Oocyst Detection.
J. Clin. Microbiol. 27(7):1490-1495.

4. **Chapman, P.A., B.A. Rush and J. McLauchlin, 1990.**
An enzyme immunoassay for detecting *Cryptosporidium* in faecal and environmental samples.
J. Med. Microbiol. 32:233-237.

5. **D'Antonio, R.G. et al., 1985.**
A waterborne outbreak of cryptosporidiosis in normal hosts.
Ann. Intern. Med. 103:886.

6. **Dubey, J.P., C.A. Speer and R. Fayer, eds., 1990.**
Cryptosporidiosis of Man and Animals.
CRC Press.

7. **Gallaher, M.M. et al., 1989.**
Cryptosporidiosis and Surface Water.
Am. J. Public Health 79(1):39-42.

8. **Hayes, E.B. et al., 1989.**
Large Community Outbreak of Cryptosporidiosis Due to Contamination of a Filtered Public Water Supply.
N. Eng. J. Med. 320(21):1372-1376.

9. **Leech, J.H., M.A. Sande and R.K. Root, eds., 1988.**
Parasitic Infections.
Churchill Livingstone.

10. **Navin, T.R. and A.M. Hardy, 1984.**
Cryptosporidiosis in Patients with AIDS.
J. Infect. Dis. 155:150.

11. **Stehr-Green, J.K. et al., 1987.**
Shedding of Oocysts in immunocompetent individuals infected with *Cryptosporidium*.
Am. J. Trop. Med. 1 Hyg 36(2):338-342.

12. **Taylor, J.P. et al., 1985.**
Cryptosporidiosis outbreak in a day-care centre.
Am. J. Dis. Child. 139:1023-1025.

13. **Ungar, BLP. 1990.**
Enzyme-linked immunoassay for detection of *Cryptosporidium* antigens in faecal specimens.
J. Clin. Microbiol., 28:2491.

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