コスモ・バイオ株式会社

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ProSpecT™ Giardia Microplate Assay

CLSI Procedure Provided by Remel Inc.

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This document does not replace the Instructions for Use (IFU). It is the responsibility of the end user to refer to the actual IFU which accompanies the product for procedural use and most recent product information.

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Procedure	ProSpecT™ Giardia Microplate Assay

Prepared by	Date Adopted	Supersedes Procedure #

Review Date	Revision Date	Signature

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This document does not replace the product insert. It is the responsibility of the laboratory to refer to the actual product insert for procedural use and most recent product information.

PRINCIPLE

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

ProSpecT Giardia Microplate Assay is a solid phase immunoassay for the detection of GSA 65. Diluted stool specimens are added to breakaway 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 labeled 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 colored reaction product. Color 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 colored reaction product develops.

SPECIMEN

Type:

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.

EQUIPMENT AND MATERIALS

Materials Required (not provided)

- Stool specimen collection containers
- Timer that measures minutes
- Wash bottle for Wash Buffer
- Distilled or deionized 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

Materials Provided:

The ProSpecT™ *Giardia* Microplate Assay includes sufficient reagents to perform 24 or 96 tests.

- Microplate strips – 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.
- Enzyme Conjugate – One dropper bottle containing 5 ml (R2458024) or 25 ml (R2458096) of horseradish peroxidase labeled mouse monoclonal anti-GSA with bovine serum and antimicrobial agents.
- Positive Control – One dropper bottle containing 4 ml of a buffered solution with inactivated Giardia antigen, and antimicrobial agents.
- Negative Control – One dropper bottle containing 4 ml of a buffered solution with a red dye, and antimicrobial agents.
- 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 – 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 deionized water. Diluted Wash Buffer is stable for 1 month when stored at 2 - 8°C.
- Color Substrate – One dropper bottle containing 12 ml (R2458024) or 25 ml (R2458096) of 3,3',5,5'-tetramethylbenzidine (TMB) in buffer.

  The Color 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 Color Substrate to the original bottle.
- Stop Solution – One dropper bottle containing 12 ml of 0.46 mol/l Sulfuric acid.
- Package Insert – 1
- Procedure Card – 1
- Transfer Pipettes – 1 bag

Storage Requirements:
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 multi-channel pipettes. If excess reagent has been poured, the excess should be discarded. Do not pour excess reagent back into the bottle.

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 colorless when read visually. If yellow color 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 color less than 2+ on the Procedure Card is present in the Positive Control, call for technical assistance.

PROCEDURE

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.
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 “A” for dilution in wells; See “B” for dilution in tubes.

A. Dilution in wells

3. **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.
4. **Preserved or Watery Unpreserved Specimens:** Mix by shaking specimen collection containers. No further preparation is necessary.
5. Add **4 drops** Negative Control to well A1. Add **4 drops** Positive Control to well B1.
6. Add **100 µl** SDB to each specimen well.
7. 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.

Proceed to Step 8

B. Dilution in Tubes

3. **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.
4. **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.**
5. 3 Add **4 drops** Negative Control to well A1.
6. 4 Add **4 drops** Positive Control to well B1.

7. 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.

Proceed to Step 8

8. **Cover** microplate and incubate at room temperature (20 - 25°C) for **60 minutes**. Begin timing after the addition of the last specimen.
9. 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.
10. Remove as much Wash Buffer as possible but do not allow the wells to dry out at any time.
11. Add **4 drops** (200 µl) of Enzyme Conjugate to each well.
12. **Cover** microplate and incubate at room temperature (20 - 25°C) for **30 minutes.**
13. Shake out or aspirate and wash each well **5 times** as in step 9.
14. Add **4 drops** (200 µl) of Color Substrate to each well.
15. **Cover** microplate and incubate at room temperature (20 - 25°C) for **10 minutes.**
16. Add **1 drop** (50 µl) Stop Solution to each well. Gently tap or vortex the wells until the yellow color is uniform. Read reactions within **10 minutes** after adding the Stop Solution.
17. Read visually or spectrophotometrically at 450 nm (single wavelength) or 450/620 to 650 nm (dual wavelength).

REPORTING RESULTS

VISUAL

- Read the test results by comparing with the reaction colors on the Procedure Card. Positive: yellow color of at least 1+ intensity Negative: colorless
- Interpretation of visual results:
  Positive: If yellow color 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 color (less than 1+) should be repeated.
  Negative: A colorless 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

- Read results at either single (450 nm) or dual (450/620 to 650 nm) wavelength.
- Read the optical density (O.D.) for the Negative Control.
- 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.
- 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)
- 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.

Limitations:

- The validity of results with the ProSpecT Giardia Microplate Assay depends on the control reaction performing as expected. See Quality Control section.
- 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 Specimens section.
- ProSpecT Giardia Microplate Assay has been classified as high complexity.