Trinity Biotech

HEp-2 MARBLOT STRIP TEST SYSTEM

Western Blot System for the detection of IgG antibody to human extractable nuclear antigens

REF 40-3020G **20 Tests**

Store kit at +2 to +8°C

Pour d'autres langues
Für andere Sprachen
Para otras lenguas
Per le altre lingue
Dla innych języków

Para outras línguas
Για τις άλλες λώσσες
För andra språk
For andre språk

www.trinitybiotech.com

INTENDED USE

For the identification of auto-antibodies to extractable nuclear antigens (ENA) utilizing a Western Blot Test System. The HEp-2 Marblot Test System is not to be used for diagnostic purposes and is intended to be used only when the actual diagnosis is based on an established method or procedure including clinical findings. The test system is For Professional Use Only. ***For Export Only***

SUMMARY AND PRINCIPLES

Until recently assays for nuclear antibodies had relied primarily upon the Antinuclear Antibody Screening Test (ANA) (1,4,12). The need to further identify these antibodies beyond the initial ANA screen has become important in the positive patient profile (13). Additional testing is needed to specifically characterize nuclear antibodies such as saline extractable nuclear antibodies (ENA) and DNA antibodies.

Historically there has been a high degree of disease correlation between antibodies to DNA and SLE patients. More recently, Sm antibodies have been significantly associated with Systemic Lupus disease flare in 50%, while exacerbation of the disease process is reported in 60% of the patients exhibiting a rise in titer. Therefore, the test for Sm antibodies demonstrate not only diagnostic, but also prognostic value (9,10).

RNP antibodies are usually associated with a greater number of diseases such as Systemic Lupus Erythematosis (SLE), Mixed Connective Tissue Disease (MCTD), Progressive Systemic Sclerosis (PSS) and possibly Sjogren's Syndrome (SS) and Rheumatoid Arthritis (RA). When RNP antibodies are present alone in significantly higher titers, it is considered specific for MCTD (7).

RNP antibodies are usually associated with a more benign disease process, with a lesser incidence of renal involvement and a more favorable prognosis (7,8). However, RNP positive patients exhibiting a more serious disorder such as nephritis have additional autoimmune antibodies such as CNA (8). SSA and SSB can be found in Sjogren's Syndrome (SS). SSA and SSB may also be found in SLE patients. SSA may be found in scleroderma patients. SCL-70 and anticentromere antibodies can be found in patients with Scleroderma (13). Jo-1 is found in polymyotisis. PCNA is found in SLE. This information is summarized in Table 1.

Table 1
CORRELATION OF NUCLEAR ANTIBODIES TO VARIOUS DISORDERS

Disorder	Antibodies
SLE	PCNA, DNA, Sm, RNP, SSB, SSA
MCTD	RNP
PSS	RNP
SS	RNP, SSB, SSA
RA	RNP
Scleroderma	SSA, SCL-70, Anti-Centromere
Polymyositis	Jo-1

PRINCIPLES OF THE TEST

The MarDx HEp-2 Marblot Test System is a Western Blot technique utilizing antigens of human HEp-2 cells which are fractionated by molecular size in the presence of sodium dodecyl sulfate (SDS) and separated by polyacrylamide gel electrophoresis. The resolved protein bands are then transferred by transblot electrophoresis to a nitrocellulose membrane. The membrane is dried, cut into strips and packaged.

Patient serum is diluted and incubated with individual HEp-2 Marblot strips. If ENA specific antibodies are present, they will bind to the corresponding ENA antigen bands. After washing the unbound serum from the strip, the bound ENA specific antibody is reacted with alkaline phosphatase conjugated anti-human IgG. The strip is then washed to remove the unbound conjugated antibody. Finally, the strip is reacted with a precipitating color developing solution which deposits a purple precipitate onto the antibody reacted antigen bands.

The MarDx Hep-2 MarBlot Strip Test System contains a Specimen Reactive Control (SRC) stripe on each strip. The SRC stripe is located approximately 3mm below the bottom edge of the strip label (Figure 1). The purpose of this control stripe is to assure that the application of human serum (test specimen) has occurred during the test procedure. If the test specimen has been applied, the SRC stripe will develop a purple band after the Color Developing Solution has been applied. The presence of the SRC stripe on a test result assures the application of test sample on each test strip.

WARNINGS

1. For Export Only.
2. Handle samples, assay strips, immunoblot controls and serum locator as if capable of transmitting an infectious agent (Biosafety Level 2). Each donor unit used in the preparation of this material was tested by an FDA approved method for the presence of the antibody to HIV as well as for HBsAg and found to be negative (were not repeatedly reactive). WARNING - POTENTIAL BIOHAZARDOUS MATERIAL Because no test method can offer complete assurance that human immunodeficiency virus (HIV), hepatitis B virus, or other infectious agents are absent, these human control reagents should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health Manual "Biosafety in Microbiological and Biomedical Laboratories", 1999. (19)
3. The preservatives used in the reagents may be toxic if ingested.
4. 10X Sample Wash Diluent/Wash Solution contains <1% Sodium Azide. Each control and conjugate component contain <0.1% sodium azide. Sodium azide is toxic if ingested and forms potentially explosive copper and lead azide compounds in waste plumbing lines. Should the reagents come in contact with copper or lead waste plumbing, flush the waste line with large quantities of water to prevent the formation of potentially explosive compounds.
5. CAUTION: all blood products should be treated as potentially infectious. Source material from which this product was derived was found negative when tested in accordance with current FDA required tests. No known test methods can offer assurance that products derived from human blood will not transmit infectious agents.
6. Each control and conjugate contains 20% glycerol, a substance that is irritating to the eyes and skin.
7. Wear suitable protective clothing, gloves and eye/face protection throughout the test procedure. Thoroughly wash hands after handling test reagents.
8. Wipe spills immediately with a laboratory approved disinfectant.
9. If substances come into contact with eyes or skin, wash immediately with plenty of water.
10. Dispose of all specimens and materials used in the MarDx Marblot procedure as biohazardous waste.

PRECAUTIONS

1. Do not dilute the conjugate beyond the stated concentration.
2. Do not deviate from the specified temperature and timing requirements as listed in the package insert for both incubation and washing steps. Deviations will significantly alter the results of this test.
3. All reagents must be brought to 20 to 25°C before performing the test procedure. Temperatures above or below the recommended range will result in substantial variation of the test results.
4. Do not interchange kit components from one kit lot with another kit lot.
5. DRY HEp-2 MARBLOT STRIPS ARE EXTREMELY FRAGILE. *HANDLE WITH CARE*.

The safety data sheet is available upon request.

WARNING
Some components of this kit contain < 0.1% sodium azide.
EUH031: Contact with acids liberates toxic gas
H302: Harmful if swallowed.
H317: May cause an allergic skin reaction.
H335: May cause respiratory irritation.
P264: Wash thoroughly with plenty of soap and water after handling.
P270: Do not eat, drink or smoke when using this product.
P280: Wear protective gloves / protective clothing / eye protection / face protection.
P301+P312: IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell.
P302 + P352: IF ON SKIN: Wash with plenty of soap and water.
P330: If swallowed, rinse mouth.
P333 + P313: If skin irritation or rash occurs: Get medical advice/ attention.
P501: Dispose of contents and container in accordance to local, regional, national and international regulations

STORAGE AND STABILITY

1. Store kit at +2 to +8°C.
2. Bring all required components to room temperature prior to use.
3. Refer to the expiration date on all reagents.

ADDITIONAL MATERIALS REQUIRED BUT NOT SUPPLIED

1. Platform rocker capable of rocking at a maximum angle of 7° ± 2° at 40 – 45 cycles per minute.
2. Plastic or blunt tipped forceps.
3. Pipettes capable of delivering 20µL, 80µL and 2.0mL.
4. Disposable pipette tips.
5. Graduated serological pipette capable of delivering 10.0mL.
6. 100mL and 1.0L graduated cylinders.
7. Laboratory timer.
8. *MarDx* Strip Incubation Tray or equivalent.

MATERIALS PROVIDED

Prod#	Description	Quantity
40-3018	HEp-2 Marblot Strip	20 ea.
40-3022L	HEp-2 WB IgG Positive Control	1x250 µL
40-3021L	HEp-2 WB Negative Control	1x250 µL
40-3025G	Alkaline Phosphatase Conjugate, Anti-Human IgG (Goat)	1x4.5 mL
40-2017	Alk. Phos. Developing Solution	1x45 mL
40-2019	10x Sample Diluent/Wash Solution	1x100 mL
40-2030	Sample Diluent/Wash Powder	1x5 g
	Result Log	1 ea.

REAGENT PREPARATION

1. Prepare 1X Sample Diluent/Wash Solution to be used in the assay from the 10X Sample Diluent/Wash Solution provided. Upon removal of the 10X Sample Diluent/Wash Solution from refrigeration, undissolved salts may be present. Allow the reagent to reach room temperature and shake the bottle to dissolve the salts. Do not dispense the reagent until all of the salts are dissolved. In order to prepare the 1X Sample Diluent/Wash Solution add 100 mL of the 10X Sample Diluent/Wash Solution to 900 mL of distilled (or deionized) water in a clean bottle. Open the Sample Diluent/Wash Solution Powder packet and add the contents of the packet to the 1X Sample Diluent/Wash Solution, mix until dissolved. Store the unused prepared solution at +2 to +8°C between uses for up to 14 days.
2. Dilute the Alkaline Phosphatase Antihuman Conjugate 1 part to 9 parts with prepared 1X Diluent/Wash Solution containing Sample Diluent/Wash Powder prior to use (see step 1 of this section). Two (2.0) mL of the diluted conjugate will be required for each specimen or control. Do not prepare more conjugate than necessary for any specific test run. THIS DILUTED CONJUGATE CAN NOT BE STORED FOR FUTURE USE.

SPECIMEN COLLECTION

Serological specimens should be collected under aseptic conditions. Avoid hemolysis by prompt separation of the serum from the clot. Serum should be stored at +2 to +8°C if it is to be analyzed within 4-7 days. Serum may be held for 3 to 6 months by storage at -20°C or lower. Lipemic or extremely hemolyzed serum should be avoided. When specimens are shipped at ambient temperatures, additions of a preservative such as 0.01% thimerosal (merthiolate) or 0.1% sodium zide is strongly recommended. The CLSI provides recommendations for storing blood specimens (Approved Standard Procedure for the Handling and Processing of Blood Specimens, H18-A2 2005). (20)

PREPARATION OF CONTROLS

The positive and negative controls are ready to use and do not require any prior dilution before addition to the reaction tray.

PREPARATION OF SPECIMENS

Specimens do not require any prior dilution before addition to the strip incubation tray.

PROCEDURAL NOTES

1. All procedural steps should be followed as written. Failure to do so may result in aberrant test results.

2. Record the Kit Lot number and expiration date on the record sheet. Use strips from each kit in consecutive, numerical order.

   Always use forceps when handling the strips.

3. Assay trays should be rocked at a maximum platform angle of 7° ± 2° at 40 to 45 cycles per minute. Position the tray so that reagents mix lengthwise along the strips.

   Caution: Proper rocking of assay trays is essential. Use of devices that do not provide rocking motion, such as orbital shakers, may adversely affect test sensitivity and result in aberrant test results.

4. Fluid in the channels must be completely decanted prior to the next rinse or reagent addition.

5. Keep the tray level when adding reagents and specimens to the channel. If necessary carefully tip the tray toward you to position the strips at the end of the channel to avoid dispensing the control directly onto the strip.

6. Add samples, conjugate, and substrate to the ends of the channels, not directly onto the strips.

7. Caution: It is extremely important to prevent cross-contamination between channels. Exercise care when dispensing, rocking and decanting fluids.

8. The Negative and Positive Controls provided are required in each run.

9. If reusable acrylic trays are used for processing the specimens, thorough cleaning of the trays is essential.

   DAILY - Rinse tray(s) thoroughly with distilled water AFTER USE.

   WEEKLY - Soak tray(s) in sodium hypochlorite* (household bleach) solution overnight. Rinse tray(s) **thoroughly** with tap then distilled water, .ANY RESIDUAL BLEACH WILL ALTER THE REACTIVITY OF THE ASSAY. **Dilute to a 10% solution with distilled or deionized water*.

TEST PROCEDURE

1. Carefully remove the required number of HEp-2 Marblot strips from the strip vial with blunt forceps. **DO NOT TOUCH STRIPS WITH YOUR HANDS.** Place the strips in channels of a strip incubation tray, one strip per channel with the number facing up. Use strips in consecutive numerical order.

2. For each control or sample to be tested, fill a channel with exactly 2.0mL Sample Diluent/Wash Solution. Add Sample Diluent slowly and directly to the space above the strip number. Visually check to make sure strip is completely wet and not partially floating on top of the buffer.

3. Allow the strips to soak for a minimum of five minutes while rocking on an appropriate platform rocker.

4. Remove the incubation tray from the platform rocker.
5. Add 80µL of each control to channels 1 and 2 in the space above the strip number. If necessary carefully tip the tray toward you to position the strips at the end of the channel to avoid dispensing the control directly onto the strip.

6. Add 20µL of each sample to the appropriately marked channel in the space above the strip number using the same technique stated in step 5.

7. Place the incubation tray on the platform rocker and incubate by rocking for 30 minutes.

8. Remove the incubation tray from the rocker platform after incubation.

9. Decant the contents by carefully tipping the incubation tray.

10. Add 2.0mL of Sample Diluent/Wash Solution to each channel of the incubation tray and incubate for 5 minutes while rocking on the platorm rocker. Completely decant the solution after the 5 minute incubation. *Repeat this process two additional times to ensure thorough rinsing of the unbound specimen from the membrane strips.* Do not use squeeze bottle to wash strips.

11. Pipette 2.0 mL of the previously prepared Alkaline Phosphatase conjugated Anti-human IgG into each channel of the incubation tray.

12. Place the incubation tray on the platform rocker and incubate by rocking for 15 minutes.

13. Remove the incubation tray from the platform rocker after incubation.

14. Decant the contents by carefully tipping the incubation tray.

15. Add 2.0mL of Sample Diluent/Wash Solution to each channel of the incubation tray and incubate for 5 minutes while rocking on the platform rocker. Completely decant the solution after the 5 minute incubation. *Repeat this process two additional times to ensure thorough rinsing of the unbound conjugate from the membrane strips.*

16. Add 2.0 mL of distilled or deionized water to each channel of the incubation tray. Allow strips to rock for one minute.

17. Decant and completely drain the distilled or deionized water from the incubation tray.

18. Add 2.0mL of Alkaline Phosphatase Developing Solution to each channel of the incubation tray.

19. Place the incubation tray on the platform rocker and incubate for at least 4-12 minutes or until the positive control has sufficiently developed. At full development of the strip, the positive control should be easily read.

20. Remove the incubation tray from the platform rocker after incubation.

21. Decant the contents by carefully tipping the incubation tray.

22. Add 2.0mL of distilled or deionized water to each channel of the incubation tray. Rock the incubation tray by hand 3-4 times. Repeat this step two (2) additional times.

23. Remove the strips from the channels while wet using blunt forceps. Place the wet strips on paper towel and allow to air dry before mounting and interpreting the data.

24. Do not expose the membrane to direct lighting for extended periods. This will cause fading of the color.

QUALITY CONTROL

1. A positive and a negative control must be included in each run.

2. Refer to the illustration and to the Result Log to identify bands in the positive control.

3. No significant bands, other then the SRC band, should be present with the negative control.

4. The Specimen Reactive Control stripe (Figure 1) must be visible on each test specimen strip.

INTERPRETATION OF RESULTS

1. Bands at the designated molecular weights are positive for Western Blot-reactive IgG antibodies. Only one of the SSA bands (60kD, 52kD) or Sm bands(29kD, 28kD) need be present to constitute a positive.

2. Specimens which have nonspecific banding, or no banding are negative for Western Blot-reactive IgG antibodies.

3. Non-specific bands may be faintly or strongly demonstrated by any specimen. Bands are most often found at the 34, 35, and 17kDa regions, but others can be present. These are presently designated as non-specific (NS) bands and are not considered diagnostically significant.

INTERPRETATION OF RESULTS (continued)

Figure 1

LIMITATIONS OF PROCEDURE

1. No standard criterion exists for the interpretation of Western Blot for autoimmune disease. Our evaluation of Western Blot is based on specific bands and number of bands present. Generally sera which contain antibodies detectable in Ouchterlony double diffusion give strong banding patterns on Western Blot. Specificity and reactivity of blot-reactive antibodies in these specimens correlate with test results of assays utilizing native, non-denatured antigens.

   It has been reported that detection of autoantibodies to the Ro(SSA) family of proteins in the sera of SLE and Sjogren's syndrome patients is dependent on source antigen, type of assay, and *heterogeneity of individual immune response* (15,18). Sera therefore may be strongly reactive in immunodiffusion and have little reactivity on Western Blot. Sera may also react strongly on Western Blot but be negative for anti-Ro(SSA) in immunodiffusion.

2. The number and location of specimen bands which may react with the MarDx HEp-2 Marblot kit may not be equivalent to banding seen with other Western Blot procedures.

3. The increased sensitivity and specificity afforded by Western Blot may cause specimens to demonstrate multiple banding patterns which vary from specimen to specimen and case to case (16).

4. Sera from normal individuals as well as autoimmune patients can react with bands of no known diagnostic significance. These bands (34, 35, 17 kDa being the most common) are usually weak in healthy individuals, but can be very strong in true autoimmune patients. While possibly an indication of an activated immune state, they are designated as non-specific because they are also commonly demonstrated in serum from a normal population.

5. In a positive Western Blot where the antigenic specificities have co- migrating locations(ro(SSA) and Scl-70), additional testing by another method may be required to distinguish between the two disease states.

6. No diagnosis should be based upon a single test procedure. All test results must consider the clinical history presented by the patient.

REFERENCES

1. Reichlin M., Maddison PJ: Soluble tissue auto-antigens which precipitate with sera of patients with connective tissue diseases. Immunopathology of the skin, 2nd 3d. (EH Beytner, TP Chorzelski, SF Bean, eds.) John Wiley and Sons, NY, pp351-362, 1979).

2. Tan EM: Auto-antibodies to nuclear antigens (ANA): Their immunobiology and medicine.Advances in Immunology ee: pp167-240, 1982.

3. Nisengard RJ: Antinuclear antibodies: significance of titers. Immunopathology of the skin, 2nd 3d. (EH Beytner, TP Chorzelski, SF Bean, eds.) John Wiley and Sons, NY, pp387-398, 1979).

4. Tan, EM et al: The 1982 revised criteria for the classification of anti-systemic lupus erythematosus.Arth & Rheum 25: 1271-1277,1982.

5. Aarden LA, Groot ER, Feltkamp TE: Immunology of DNA 111 - Crithidia luciliae, a simple substrate for the determination of anti-ds-DNA with the immunofluorescence technique. Annals NY Acad Sci 254: 505-515, 1975.

6. Sharp GC: Anti-nRNP and Anti-Sm antibodies. Arth & Rheum 25: 757-760, 1982.

7. Levo y, Lotan C: Significance of anti-ENA, Sm, and RNP antibodies in patients with antinuclear antibodies. Isr J Med Sci 18: 287-289, 1982.

8. Reichlin M, Mattioli M: Correlation of a precipitin reaction to an RNA protein antigen and a low prevalence of nephritis in patients with systemic lupus erythematosus.New Eng J Med 286: 908-911, 1972.

9. Barbara FA, Andrews BS, Davis JS, Taylor RP: Antibodies to Sm in patients with systemic lupus erythematosus. Arth & Rheum 24: 1236-1244, 1982.

10. Winn DM, Wolfe JF, Lindburg DA, Fristoe FH, Kinsland L, Sharp GC: Identification of a clinical subset of antigen.Arth & Rheum 22: 1334-1337, 1979.

11. Gaudreau A, Amor B, Kahn MF, Rycbewacrt A, Sany J, Peltier AP: Clinica l Significance of antibodies to extractable nuclear antigen. Ann Rheum Dis 37: 321-327, 1978.

12. Harmon CE, Deng JS, Peebles CL, Tan EM: The importance of Tissue Substrate in the SS-a/RO Antigen-Antibody System. Arth & Rehum 27: 2, 1984.

13. Miyachi K, Haito M, Macono Y: ea al. Sialographic study in patients with and without antibodies to Sjorgren's Syndrome A (RO).J. Rheum 10: 387-396, 1983.

14. Nakamura, Peebles, Rubin, Modern, Tan Auto Antibodies to Nuclear Antigens.American Society of Clinical Pathologists Press, 2nd ed., 1985.

15. Rader, Michael D., Codding, Chris, Reichlin, Morris. Arth & Rheum. Vol. 32: No.12, 1563-1571, 1989.

16. Ben-Chetrit, Eldad; Fox, Robert L.; Tanc Eng.Arth & Rheum. Vol. 33: No. 3, 349-355, 1990.

17. McNeilge, L. Jane; MacMillan, Elizabent M.; Whittingham, Senga F. Journal of Immunology. Vol. 145: No. 11, 3829-3835, 1990.

18. Arnett, Frank C. etal.Arth & Rheum. Vol 32: No. 4, 413-419, 1989.

19. Centers for Disease Control/National Institutes of Health (CDC-NIH) Manual. 1999. In: *Biosafety in Microbiological and Biomedical Laboratories*, 4th Edition, U.S. Dept. of Health and Human Services, Public Health Service.

20. Clinical Laboratory Standards Institute (CLSI) 2005. Procedures for the Handling and Processing of Blood Specimens; Approved Guideline – Second Edition. CLSI Publication H18-A2.

Consult Instructions for Use

REF	Product Number
LOT	Lot Number
IVD	*In Vitro* Diagnostic Medical Device
EC REP	Authorized Representative in the European Community
	Use By
	Caution, consult accompanying documents
	Temperature limitation
	Manufacturer
	WARNING
CONTROL -	Negative Control
CONTROL +	Positive Control
CONJ	Conjugate
SUBS	WB Color Developer
DIL 10X	10X Sample Diluent/Wash Solution
DIL PDR	Sample Diluent/Wash Powder
STRPS	Western Blot Strips

EC REP-Trinity Biotech plc.
IDA Business Park
Bray, County Wicklow, Ireland
Phone: +353-1-276-9800
Fax: +353-1-276-9888
Web: www.trinitybiotech.com

Manufactured By
MarDx Diagnostics,Inc.
A Trinity Biotech Company
5919 Farnsworth Court
Carlsbad, CA 92008
Phone: 800-325-3424
Fax: 760-929-0124

40-3020G-29-Rev. 5
05/2015