LIGHT **DIAGNOSTICS™**

ECHOVIRUS 30 MONOCLONAL ANTIBODY

For the Presumptive Identification of Echovirus 30 Isolates in Cell Culture

REF **3315**

IVD CE

EMD Millipore Corporation
28820 Single Oak Drive Temecula, CA 92590 • United States
Tel. : +1 (951) 676-8080 • Fax : +1 (951) 676-9209
www.millipore.com

EC REP
MDSS GmbH
Schiffgraben 41
30175 Hanover, Germany

This page left blank intentionally.

Intended Use

Light Diagnostics™ Echovirus 30 Monoclonal Antibody is a type-specific reagent intended for use in indirect immunofluorescence screening for the presumptive identification of echovirus 30 obtained in cell culture and not intended for testing directly on human specimens.

IVD

Background and Clinical Significance

Enteroviruses, such as echoviruses, are classified to be in the picornavirus family, pico [small] + RNA [ribonucleic acid] + virus. Picornaviruses are among the smallest and simplest ribonucleic acid containing viruses known (1). The RNA for many enteroviruses have now been cloned and complete genomic sequences have been obtained. The RNA from all sequenced enteroviruses are similar in length, about 7400 nucleotides, and have identical organization (1).

The human alimentary tract is the predominant site of enterovirus replication and these viruses were first isolated from enteric specimens. These viruses are the cause of paralytic poliomyelitis, aseptic meningitis-encephalitis, myocarditis, pleurodynia, hand-foot-and-mouth disease, conjunctivitis, and numerous other syndromes associated with extra-intestinal target organs. There are 67 numbered types of enteroviruses in the enterovirus family (1): three polioviruses, twenty-three coxsackieviruses A, six coxsackieviruses B, thirty-one echoviruses, and four other enteroviruses.

Enteroviruses, including echoviruses and coxsackieviruses, have been reported as the major etiologic agents of aseptic meningitis (2). Clinical syndromes associated with infections by each type of enterovirus have also been reported (3). Echoviruses can cause aseptic meningitis, paralysis, encephalitis, ataxia, Guillain-Barre' syndrome, exanthema and respiratory disease. Echoviruses have also been associated with diarrhea, epidemic myalgia, pericarditis, myocarditis and hepatic disorders.

Establishing an association between an enterovirus and a particular disease in a patient requires laboratory confirmation of infection, usually by either isolation of the virus or documentation of a specific serologic response in a properly timed specimen. Detailed descriptions of principles and procedures for diagnosis of enterovirus infections have been published (4-7). Cell culture

techniques have made the accurate detection of enteroviruses possible (8-10). The identification of the enterovirus isolates help prevention, treatment and understanding of the infectious diseases, and even discovery of new virus isolates. The typing of enterovirus isolates is generally accomplished by neutralization with type-specific pools of immune sera (11). This method is time consuming (7 days or more) and expensive. As an alternative, typing of enteroviruses with type-specific monoclonal antibody and/or group-specific monoclonal antibody pool(s) by Indirect Immunofluorescence is potentially more rapid and less expensive (12 - 18).

Test Principle

Light Diagnostics™ Echovirus 30 Monoclonal Antibody (Echovirus 30 MAb) can be used to identify echovirus 30 isolates in cell culture using an indirect immunofluorescence assay (IFA). The monoclonal antibodies provided will bind to the type-specific echovirus 30 antigen present on the cell culture slide. Unbound monoclonal antibody is removed by rinsing with phosphate-buffered saline (PBS). A secondary FITC (fluorescein isothiocyanate)-labeled antibody is then added which will bind to the antigen-antibody complex. Unbound secondary antibody is removed by rinsing with PBS. FITC exhibits an apple-green fluorescence when illuminated by ultraviolet light allowing visualization of the complex by microscopy. A positive result is indicated by cell fluorescence. Uninfected cells stain a dull red if Evans Blue counterstain is used in the FITC-labeled secondary antibody or used elsewhere in the procedure.

Reagent Provided

Echovirus 30 Monoclonal Antibody - REF 3315.

One 1 mL dropper vial containing mouse IgG_{2a} monoclonal antibody against echovirus type 30, protein stabilizer and 0.1 % sodium azide (preservative).

Materials Required But Not Provided

- Acetone, reagent grade; stored in glass
- Distilled water or deionized water
- Sodium hypochlorite solution, 0.05% (1:100 dilution of household bleach)

- Sterile tissue culture tubes or shell vials with 12 mm coverslips containing monolayer of cell line appropriate for growth of enteroviruses
- Tissue culture media such as RPMI or Eagle's Minimum Essential Medium (EMEM) with fetal bovine serum (FBS) and antibiotics, or equivalent
- Viral transport medium which is non-inhibitory to enterovirus
- 0.1N NaOH
- 0.1N HCl
- Microscope slides, non-fluorescing
- No. 1 cover slips
- Negative and positive control slides
- Anti-Mouse IgG:FITC Conjugate REF 5008
- Normal Mouse Antibody such as REF 5014 as negative control
- Phosphate-buffered saline (PBS, 0.01 M pH 7.1-7.4 with 0.085% NaCl and 0.1% sodium azide), REF 5087
- 0.05% Tween® 20 /0.1% Sodium Azide Solution (optional), REF 5037
- Aspirator device with disposable sterile Pasteur pipettes
- Centrifuge capable of 700-950 x g with biohazard buckets and adapters for shell vials
- Fluorescence microscope with appropriate filter combination for FITC (excitation peak = 490 nm, emission peak = 520 nm) with 100x, 200x, 400x magnification (dry objective)
- Forceps
- Humid chamber
- Incubator, 37 ± 1°C
- Syringe filter, 0.45 micron
- Ultrasonic water bath
- Vortex mixer or sonicator
- Mounting Fluid REF 5013
- Echovirus Control Slides REF 5074

Warning and Precautions

- The performance of **Light Diagnostics™** Echovirus 30 MAb has not been determined on direct specimens.
- Sodium azide, present in the reagent, may react with lead and copper plumbing to form potentially explosive metal azides. When disposing of solutions that contain sodium azide, flush plumbing with a large volume of water to prevent build-up
- Handle all specimens and materials coming in contact with them as potentially infectious materials. Decontaminate with 0.05% sodium hypochlorite prior to disposal.
- Avoid contact with Evans Blue if present in any reagent as it is a potential carcinogen. If skin contact occurs, flush with large volumes of water.
- Do not mouth pipette reagents.
- Do not allow shell vials or slides to dry at any time during the staining procedure.
- Pooling or alteration of any reagent may cause erroneous results.
- Acetone is extremely flammable and harmful if swallowed or inhaled. Keep away from heat, sparks or flame. Avoid breathing vapor. Use adequate ventilation.
- Mounting Fluid REF 5013 contains a fluorescence enhancer that may be destructive to mucous membranes. Avoid direct skin or mucous membrane contact. If contact occurs, flush with large volumes of water.
- Slides prepared too early (<25% CPE) or too late (>95% CPE) can be difficult to read and can lead to false negatives.

Stability and Storage

When stored at 2° to 8°C, the monoclonal antibody is stable up to the expiration date printed on the label. Do not freeze or expose to elevated temperatures. Discard any remaining reagent after the expiration date.

Specimen Collection

While procedures vary from laboratory to laboratory, some considerations on how to process specimens for enterovirus analysis are offered. The specimen type and its processing will depend on the clinical status of the patient and the laboratory request of the consulting physician. Specimens for enterovirus isolation should be transported on wet ice or cold packs and cultured as soon as possible. If storage is necessary, store at 2° to 8°C for up to 48 hours. If longer storage is necessary, store frozen at -70°C in appropriate media (19).

Specimen Processing

Body Fluids - For fluid specimens such as cerebrospinal fluid (CSF) inoculate 0.2 - 0.5 mL undiluted sample in each culture vessel.

Swabs - Specimens such as nasopharyngeal, throat or eye swabs in transport media should be agitated or vortexed to dislodge cells from the swab. Inoculate 0.2 - 0.3 mL in each culture vessel.

Fecal Material - If solid fecal matter is present, vortex in 2 - 5 mL of viral transport media or PBS. Discard the swab into sodium hypochlorite solution. Centrifuge at 950 x g for 10 minutes. If necessary, filter the supernatant through a 0.45 micron syringe filter to clarify. Inoculate 0.1 - 0.3 mL in each culture vessel.

Solid Tissues - Take small pieces of tissue specimen and place in 2 - 3 mL viral transport media. Grind tissue with tissue grinder. Centrifuge gently to sediment debris. Inoculate 0.1 - 0.2 mL supernatant in each culture vessel.

Inoculation Procedure

1. Immediately prior to inoculation of specimen, examine cell cultures for proper morphology.
2. Add appropriate volume of inoculum as indicated above to each of the culture vessels.
3. Place in a 37 ± 1°C incubator.

Test Procedure

Preparation of Slides:

1. Examine the monolayer daily for cytopathic effect (CPE). When the CPE is > 25%, aspirate the medium from the culture and gently rinse the monolayer 3 times with 1 mL PBS.

   ***Note**: Reagents containing sodium azide or surfactant such as Tween 20 should not be used in viral isolation procedures.*

2. Add 0.5 mL of PBS and scrape the culture vessel to remove the cell monolayer, resuspending the cells into the PBS.
3. Centrifuge the cell suspension at 250 x g for 10 minutes at room temperature.
4. Resuspend cell pellet in 0.1 - 0.2 mL PBS and use to make cell spots in 6 - 8 mm slide wells and allow the slide to air dry completely.
5. Fix the slide in chilled (2° to 8°C) acetone for 10 minutes.
6. Remove the slide from the acetone and allow to air dry completely.
7. The slide should be stained as soon as possible. If storage is necessary, the slides should be kept at ≤ -20°C, with desiccant.

Suggested Indirect Immunofluorescence (Staining) Procedure:

1. Allow the acetone fixed control slide and/or test slide and reagents to equilibrate to room temperature.

   ***Note:** Do not allow slides to dry at any time during the staining procedure.*

2. Add sufficient monoclonal antibody or normal mouse antibody (Neg control reagent) to cover the cells; 1 drop for cell spots and 4-6 drops for shell vials.
3. Incubate the slide at 37°C for 30 minutes in a humid chamber.
4. Rinse the slide gently with a squirt bottle of PBS/Tween 20 for 10-15 seconds to remove excess monoclonal antibody solution, taking care to direct the stream away from the well. For shell vials: aspirate reagent from vial and gently wash each shell vial 3 times with 1mL PBS/Tween 20.

5. Place slide in a staining dish or Coplin jar (with slide holder or equivalent) and cover with PBS. Rinse 5 to 10 minutes. Gently agitate with magnetic stirring bar or by hand.
6. Shake off excess reagent from the slide and carefully dry the area surrounding the cell spot.
7. Add sufficient FITC-labeled Anti-Mouse IgG Conjugate REF 5008 or equivalent to cover the cells; 1 drop for cell spots and 4-6 drops for shell vials.
8. Repeat steps 3 to 5.
9. Mount under a coverslip using an aqueous Mounting Medium pH 8.5, REF 5013 or equivalent. For shell vials: Aspirate PBS/Tween from shell vials. Raise each coverslip using a bent needle affixed to a small syringe and carefully remove with forceps. Mount each coverslip CELL SIDE DOWN on a glass slide with Mounting Fluid.
10. Wipe excess fluid from the edges of the slide.

    Note: *For best results, read slides immediately after preparation. If slides are to be stored after staining, store at 2° to 8°C, in a secure container in the dark.*
11. Examine slides, using a fluorescence microscope at 100-200x for cells exhibiting fluorescence. Detailed examination may be carried out at 400x magnification.

    Note: *Performance of the fluorescence microscope is of critical importance in achieving satisfactory test results. While objectives, bulb intensity and wattage, and filters may affect results, use of a positive control will verify functioning of reagents, culture methodology and microscope.*

Quality Control

Positive and negative controls should always be included with each test to assure proper performance of the procedure for the identification of echovirus 30 isolate(s). The positive control well should show cells exhibiting apple-green fluorescence in the nucleus and/or cytoplasm. The negative control well should show cells staining a dull red color if Evans Blue counterstain is used.

Note*: Scan the entire coverslip or slide well for the fluorescent apple-green color present in enterovirus infected cell nuclei.*
Positive controls should be prepared with the appropriate echovirus isolates or with known positive clinical isolates. Negative control can utilize known laboratory negative samples. Echovirus Control Slides REF 5074 with echovirus positive and negative wells are available from EMD Millipore Corporation. Live echovirus for positive control can be obtained from the American Type Tissue Culture Collection (ATCC®), Manassas, VA.

Caution: *Fluorescence staining of cell fragments, often due to the trapping of the conjugate in such debris, should be ignored. If positive and negative controls cannot be clearly distinguished, the test should be considered invalid and should be repeated after reviewing and making corrections suggested in the "Troubleshooting" section.*

Limitations

- Occasional low intensity background staining may occur with some viral isolates, the specific Echovirus 30 MAb always exhibited significantly greater intensity of staining.
- This monoclonal antibody has not been characterized as to the particular structural protein antigen or epitope detected on the echovirus 30 virus.
- The type and condition of the instrumentation used will influence the visual appearance of the image obtained. The endpoint reaction may vary due to the type of microscope employed, the light source, age of the bulb, filter assembly and filter thickness, differences in sensitivity of the antigen substrate, or the assay procedure used. Each laboratory should establish its own criteria for reading of the endpoints using appropriate controls.
- Since the monoclonal antibody has been prepared using a prototype strain, it may not detect all antigenic variants or new strains of echovirus 30.
- Monoclonal antibodies may fail to detect strains of echovirus 30 which have undergone minor amino acid changes in the target epitope region.

Expected Values

EMD Millipore Corporation was unable to determine a prevalence rate based on our studies. The most frequently reported enterovirus types over the past twenty years have been coxsackieviruses A9, A16, B2, B3, B4, B5 and echoviruses 4, 6, 9, 11, and 30 (20, 21). These serotypes generally constitute about 65% of all enteroviruses isolated each year. In any single year the predominance of each virus may be more marked, or another type may increase in frequency for a time.

Frequency of enterovirus infections are affected by patient age, condition, the climate and the time of year. Most of the reported cases are in young children. Climate appears to be an important factor in the circulation and prevalence of enteroviruses. In temperate climates, enteroviruses are generally present at low levels in the winter and spring, but are isolated far more commonly during the summer and fall. Even in the United States, healthy children in the southern cities harbor a greater abundance of enterovirus than do those of comparable age in the northern cities (20).

Echovirus 30 has averaged about 5.95% (range 0.5% - 19.9%) of the non-polio enteroviruses reported isolated in the United States each year since 1970 (20).

Interpretation of Results

A **positive** reaction is indicated by a **bright apple-green** fluorescence in the nucleus and/or cytoplasm of the infected cells. Positive staining for echovirus 30 is represented by the presence of at least 2 or more intact cells exhibiting specific fluorescence. A **negative** reaction is indicated by the absence of fluorescence and presence of a **dull red** color due to the Evans Blue counterstain, if used. <u>All **positive** results should be confirmed by neutralization with type-specific pools of immune sera</u>. A positive result should be reported as “isolate presumptive for echovirus 30, confirmation to follow”.

A negative result does not rule out echovirus 30 infection. The negative result may be due to a variety of factors such as: inadequate sample, improper specimen collection and handling, improper culture technique, or other factors mentioned in the "Troubleshooting" section. All negative results should be reported as "No virus observed" or "No echovirus 30 isolated, further studies to follow". It is useful to examine the negative cells prior to the positive cells to determine if there is non-specific staining.

Specific Performance Characteristics

Specificity and Cross Reactivity:

The Monoclonal Antibody against echovirus 30 correctly identified the echovirus 30 prototype strains and previously typed viral isolates provided and tested by the Center for Disease Control (CDC), and three other clinical virology laboratories.

In these studies, monoclonal antibodies directed against enteroviral antigens (both blends of different serotypes and type specific reagents) were used to test viral isolates by three external clinical virology laboratories. The clinical labs were located on the West Coast, Southwest and southern regions of the United States. Specimens were obtained from patients whose ages ranged from infants to elderly. A wide variety of specimens were cultured including fluids (CSF and urine), swabs (nasal, throat, nasopharyngeal and eye), fecal samples and rectal swabs. The specimens were inoculated into culture and were first screened by the MAb blends and then typed by IFA with the individual monoclonal components. All the viral isolates were confirmed by conventional neutralization (22). Every lab in the study used anti-Mouse IgG:FITC Conjugate REF 5008 as the second antibody for all or part of the study. The West Coast lab also used Goat anti-Mouse Ig Conjugate from another commercial source.

The Echovirus 30 MAb did not react with other viruses including coxsackieviruses A9, A10, A14, A16, A24, A24v or coxsackieviruses B1 - 6. The Echovirus 30 MAb did not react with other echoviruses including echo 1, echo 2, echo 3, echo 4, echo 5, echo 6, echo 7, echo 9, echo 11, echo 11', echo 13, echo 14, echo 15, echo 16, echo 18, echo 20, echo 21, echo 22, echo 25, echo 29, echo 32, and echo 34. No reactivity was found with each enterovirus 70 and 71, polio 1, polio 2, polio 3, herpes simplex virus (HSV-1 or HSV-2), adenovirus or rhinovirus isolates tested. In addition, control slides of uninfected host cell cultures and other pathogens such as parainfluenza 1, 2, & 3, influenza A & B, respiratory syncytial virus (RSV), cytomegalovirus (CMV), human herpes virus - 6 (HHV-6), varicella-zoster virus (VZV), mumps, measles, chlamydia, Epstein-Barr virus - viral capsid antigen (EBV-VCA), and adenovirus were tested, and no significant cross reactivity with the Echovirus 30 MAb was found.

Echovirus 30 MAb exhibited occasional low intensity background staining with some viral isolates, however, with echovirus 30 isolates it exhibited significantly greater intensity of staining. The monoclonal antibodies have been titered to minimize any nonspecific reactivity.

Clinical Evaluation:

Monoclonal antibodies directed against enteroviral antigens (both blends of different serotypes and type specific reagents) were used to test viral isolates by three external clinical virology laboratories. The viral isolates, confirmed by conventional neutralization (22), were first screened by the blends and then typed with the individual monoclonal components. A summary of the combined studies comparing the standard neutralization results and the **Light Diagnostics™** Indirect Fluorescence Assay with the Echovirus 30 MAb is shown below.

Positive by Neutralization:	161
Light Diagnostics™ IFA Positive:	149
Relative Sensitivity:	92.6 %
95% Confidence Interval Sensitivity:	87.3 % - 96.1 %

Negative by Neutralization:	652
Light Diagnostics™ IFA Negative:	650
Relative Specificity:	99.7%
95% Confidence Interval Specificity:	98.9 % - 100 %

With the exception of 2 of 33 echovirus 6 isolates *no other significant cross reactivity* with any other clinical isolate was noted. However, the Echovirus 30 MAb failed to recognize 12 of 161 echovirus 30 isolates tested. This seems to indicate that there may be a variant strain or a low percentage of echovirus 30 isolates which do not express the antigen recognized by the Echovirus 30 MAb. Yet the above results indicate that the Echovirus 30 MAb is very sensitive and specific.

(95% Confidence Intervals were calculated by the Exact Method (23).)

Troubleshooting

Specimen preparation is technique dependent and may affect the results obtained. In order to resolve any performance questions, all steps in the process must be analyzed.

A marked decrease in fluorescence may indicate:

1) Reagent deterioration,
2) Microscopy problems or
3) Other equipment or technique effects.

- Verify expiry date for all reagents used.
- If reagents are in-dating, verify microscope performance; re-read positive control.
- If problem is still not determined, verify all equipment operation as per package insert and repeat test.

Contact **EMD Millipore Corporation** - Technical Service at (800) 437-7500 for United States and Canada or (951) 676-8080. For additional assistance, visit www.millipore.com/offices.

References

1. Morens, D.M., M. A. Pallansch, and M. Moore, 1991. Polioviruses and other enteroviruses. *In* Belshe, (ed.) Text Book of Human Virology, Mosbey Year Books, pp. 427-497.
2. Connolly, K.J. and S. M. Hammer, 1990. The acute aseptic meningitis syndrome. Infect. Dis. Clin. North Am., 4:559-622.
3. Melnick, J.L. 1990. Enteroviruses. *In* B. N. Fields, and D. M. Knipe, (eds.) Virology, 2nd. ed. New York: Raven Press, pp. 549-605.
4. Melnick, J. L., H. A. Wenner, and L. Rosen, 1964. The Enteroviruses. *In* Lennette, E.H., and Schmidt, N.J. (eds.) Diagnostic Procedures for Viral and Rickettsial Diseases, 3rd ed. Washington DC: American Public Health Association, pp. 194-242.
5. Melnick, J.L., H. A. Wenner, and C. A. Phillips, 1979. Enteroviruses. In Lennette, E.H. and Schmidt, N.J. (eds.). Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infections, 5th ed. Washington DC: American Public Health Association, pp. 471-534.
6. Schmidt, N.J. and E. H. Lennette, 1973. Advances in the serodiagnosis of viral infections. Prog. Med. Virol. 15:244-308.
7. Wenner, H.A. 1964. Outline of laboratory procedure for the diagnosis of enterovirus infections. *In* E. H. Lennette, and N. J. Schmidt, (eds.). Diagnostic Procedures for Viral and Rickettsial Diseases, 3rd ed. Washington DC: American Public Health Association, pp. 243-258.
8. Menegus, M.A. 1990. Enteroviruses. *In* A. Balows, A., W. J. Hausler, Jr., H. O. Isenberg, and H. J. Shadomy, (eds.). Manual of Clinical Microbiology, 5th ed, Washington DC: American Society for Microbiology, pp. 943-947.
9. Salmon, V.C., E. W. Taggart, and J. C. Overall, Jr. 1992. Enterovirus isolation: Shell vial centrifugation vs. tube culture. Abst. 92nd Mtg. Am. Soc. Microbiol. C-157:446.
10. Wildin, S. and T. Chonmaitree, 1987. The importance of the virology laboratory in the diagnosis and management of viral meningitis. Am. J. Dis. Child. 141:454-457.

11. Lim, K.A. and M. Benyesh-Melnick, 1960. Typing of virus by combination of antiserum pools. Application to typing enteroviruses. J. Immunol. 84:309-317.

12. Leonardi, G.P., P. Costello, 1994. Use of monoclonal blends in the identification of enteroviral aseptic meningitis. Current Microbiology. 28:49-52.

13. Bastis, D., *et al.* April, 1993. An evaluation of a panel of monoclonal antibodies for the identification of enterovirus. *In* Program and Abstracts of the Ninth Annual Clinical Virology Symposium and Pan American Society for Clinical Virology.

14. Minnich, L., *et al.* April, 1993. Echovirus type II (E11) infection diagnosed by staining of nasopharyngeal cells using a commercially available monoclonal antibody (MAb E11) in an indirect immunofluorescence procedure. *In* Program and Abstracts of the Ninth Annual Clinical Virology Symposium and Pan American Society for Clinical Virology.

15. Bastis, D., *et al.* April, 1993. identification of enterovirus isolates by monoclonal antibodies. *In* Program and Abstracts of the Ninth Annual Clinical Virology Symposium and Pan American Society for Clinical Virology.

16. Schnurr, D., S. Yagi, and R. Devlin, April, 1993. Immunofluorescent typing of group B coxsackieviruses with monoclonal antibodies. *In* Program and Abstracts of the Ninth Annual Clinical Virology Symposium and Pan American Society for Clinical Virology.

17. Schnurr, D., S. Yagi, and R. Devlin, April, 1993. Typing of enteroviruses using monoclonal antibodies. *In* Program and Abstracts of the Ninth Annual Clinical Virology Symposium and Pan American Society for Clinical Virology.

18. Young, S.A., B. Strong, and R. Radloff, April, 1993. Utility of genus and type specific monoclonal antibodies in the identification of enteroviruses by immunofluorescence. *In* Program and Abstracts of the Ninth Annual Clinical Virology Symposium and Pan American Society for Clinical Virology.

19. Zeichhardt, H. 1992. Enteroviruses including hepatitis A virus. *In* S. Specter, and G. Lancz, (eds.). Clinical Virology Manual, 2nd ed. Elsevier, pp. 341-360.

20. Fields, Bernard N., *et al.* 1990. Fields Virology, 2nd ed., Raven Press, pp. 577-583.

21. Rotbart, Harley, editor, 1995. Human Enterovirus Infections, American society of microbiology, pp. 274-275.

22. Data regarding the neutralization reagents used in these studies are available upon request from Chemicon International, Inc.

23. Confidence Interval Analysis (CIA) Microcomputer Program, Version 1.1. British Medical Journal and M. J. Gardner, 1989, 1991.

Glossary of Symbols

Symbol	Used for	Symbol	Used for
	Catalog number		Use by YYYY-MM-DD or YYYY-MM
	Manufacturer		Authorized representative in the European Community
	Caution, consult accompanying documents		Contains sufficient for <n> tests
	*In vitro* diagnostic medical device		Temperature limitation
	Consult instructions for use		Biological risks
	Control		Negative control
	Positive control		

Warranty:
EMD Millipore warrants that its products will meet their applicable published specifications when used in accordance with their applicable instructions for the expressly stated duration. This warranty does not extend to any product which has been subjected to misuse, neglect, or to use in violation of instructions furnished by EMD Millipore. For products EXPRESSLY SOLD FOR USE IN FOOD, DRUG OR COSMETIC APPLICATIONS, EMD Millipore guarantees that no such product is adulterated or misbranded within the meaning of the Federal Food, Drug and Cosmetic Act (the "Act") or within the meaning of any other applicable law in which the definitions of adulteration or misbranding are substantially the same as those contained in the Act, as such laws are constituted and effective at the time of shipment, or is an article which may not, under Sections 404 or 505 of the Act, be introduced into interstate commerce.

EMD MILLIPORE MAKES NO OTHER WARRANTY, EXPRESS OR IMPLIED, AND EXPRESSLY DISCLAIMS WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR A PARTICULAR PURPOSE. The warranty provided herein and the data, specifications and descriptions of EMD Millipore products appearing in EMD Millipore's published catalogues and product literature may not be altered except by express written agreement signed by an officer of EMD Millipore. Representations, oral or written, which are inconsistent with this warranty or such publications are not authorized and if given, should not be relied upon.

In the event of a breach of the foregoing product warranty, EMD Millipore's sole obligation shall be to repair or replace, at its option, the applicable product or part thereof, provided the customer notifies EMD Millipore promptly of any such breach. If after exercising reasonable efforts, EMD Millipore is unable to repair or replace the product or part, then EMD Millipore shall refund to the customer all monies paid for such applicable product or part.

EMD Millipore warrants that it shall provide services in a professional and workmanlike manner and that it will attempt in good faith to perform the services to Customer's reasonable satisfaction. **EMD MILLIPORE MAKES NO OTHER WARRANTY EXPRESS OR IMPLIED.** If EMD Millipore breaches this warranty, then Customer shall be entitled to a refund of up to the amount paid for such services. The above represents Customer's sole remedy for breach of this services warranty.

Buyer assumes all risk and liability for loss, damage or injury to persons or to property of Buyer or others arising out of the presence or use of the products or EMD Millipore's provision of services. Except as expressly provided otherwise herein, EMD Millipore shall not indemnify nor be liable to Buyer, Buyer's customers, successors, or to any person or entity for any claims, damages or losses arising out of the sale or use of products or the provision of services, where liability is premised upon any theory including, but not limited to, warranty, negligence or strict liability. **EMD MILLIPORE SHALL NOT IN ANY EVENT BE LIABLE FOR INCIDENTAL, CONSEQUENTIAL, INDIRECT, EXEMPLARY OR SPECIAL DAMAGES OF ANY KIND RESULTING FROM ANY USE OR FAILURE OF THE PRODUCTS OR SERVICES INCLUDING, WITHOUT LIMITATION, LIABILITY FOR LOSS OF USE, LOSS OF WORK IN PROGRESS, LOSS OF REVENUE OR PROFITS, OR ANY LIABILITY OF BUYER TO A THIRD PARTY. THE TOTAL LIABILITY OF EMD MILLIPORE UNDER THESE TERMS AND CONDITIONS OF SALE SHALL NOT EXCEED THE PURCHASE PRICE OF THE PRODUCTS OR SERVICES INVOLVED.** All claims must be brought within one (1) year of delivery, regardless of their nature.

Tween® is a registered trademark of ICI Americas Inc.

ATCC® is a registered trademark of the American Type Culture Collection Corporation.

3315

March 2019
Revision 2.0; 3315MAN