

Monoclonal Technologies, Inc.

m-TECH™

6095 Lake Forrest Drive Ste 170
Atlanta, GA. 30328



TOLL FREE (U.S.): 1-888-683-2414

INTERNATIONAL: 1-770-521-1960

e-mail: sales@4m-tech.com

"Immunochemicals for Research"

DFA Staining Protocol for Legionella

STEP 1: **(ONLY NEEDED IF PREPARING SLIDES NOT PURCHASED COMMERCIALY)**

SOAK SLIDES IN A CRITICAL CLEANING SOLUTION SUCH AS MICRO (AVAILABLE FROM COLE-PALMER) FOR SEVERAL HOURS OR OVERNIGHT. RINSE SLIDES WITH COPIOUS AMOUNTS OF STERILE DISTILLED WATER. COMPLETELY AIR DRY AND STORE IN AN AIRTIGHT CONTAINER.

STEP 2:

APPLY CULTURE OR ANTIGEN SUSPENSION TO SLIDE (FOR CLINICAL SPECIMENS FOLLOW THE PROTOCOLS OUTLINED IN "DETECTION OF LEGIONNAIRES' DISEASE BACTERIA IN CLINICAL SPECIMENS BY DIRECT IMMUNOFLUORSCENCE"). THE CULTURE SUSPENSION IS USUALLY IN 1% FORMALIN IN 0.85% NaCl, A CONCENTRATION TO GIVE A SLIGHT TURBIDITY (MCFARLAND #1 OR LESS)

STEP 3:

ALLOW THE ANTIGEN SUSPENSION TO AIR DRY THEN LIGHTLY HEAT FIX THE SMEAR(S).

STEP 4:

OVERLAY THE ANTIGEN WITH THE APPROPRIATE DFA CONJUGATE (FOR A 5mm WELL THIS IS APPROXIMATELY 20 MICROLITERS). BE CERTAIN THE ENTIRE WELL IS COVERED TO AVOID AREAS OF DRIED CONJUGATE ON THE SMEAR.*. ALWAYS PLACE THE NEGATIVE CONTROL CONJUGATE ON THE SMEAR(S) NEAREST THE LABELED END OF THE SLIDE. THIS IS SO THE STREAM OF BUFFER OR WATER WILL NOT WASH STAINED ORGANISMS ONTO THE NEGATIVE CONTROL SMEAR(S).

STEP 5:

PLACE THE SLIDES(S) IN A MOIST CHAMBER FOR 30 MINUTES AT ROOM TEMPERATURE.

STEP 6:

REMOVE EXCESS CONJUGATE BY HOLDING THE SLIDE LEVEL, BUT PERPENDICULAR, AND TAPPING IT AGAINST A TOWEL. QUICKLY AND GENTLY RINSE THE SEMARS WITH A STREAM OF PBS (0.01 M@ Ph 7.6) FROM A WASH BOTTLE WHILE KEEPING THE SLIDE ROUGHLY HORIZONTAL WITH THE LONG EDGE TIPPED SLIGHTLY DOWNWARD. PREVENT THE SPECIFIC CONJUGATE FROM COMING INTO CONTACT, EVEN MOMENTARILY, WITH THE CONTROL SMEAR.

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Toll Free (U.S.): 1-888-683-2414 / Phone: 770-521-1960 / Fax: 770-521-1930 /
E-mail: sales@4m-tech.com / <http://www.4m-tech.com>

DFA STAINING PROTOCOL CONTINUED

STEP 7:

IMMERSE SLIDE IN A PBS FOR 10 MINUTES. USE A SEPARATE PBS CONTAINER FOR EACH SLIDE TO PREVENT CROSS -CONTAMINATION WITH ORGANISMS THAT MAY WASH OFF LDB POSITIVE SLIDES.

STEP 8:

WASH EACH SLIDE IN STERILE DISTILLED WATER FROM A WASH BOTTLE. TAP THE SLIDE AGAINST A TOWEL TO REMOVE MOST OF THE WATER.

STEP 9:

AIR DRY COMPLETELY.

STEP 10:

ADD 3-4 DROPS OF BUFFERED GLYCEROL (Ph 9.0 to 9.5) AND A #1 (CORNING) COVER SLIP TO THE SLIDE.