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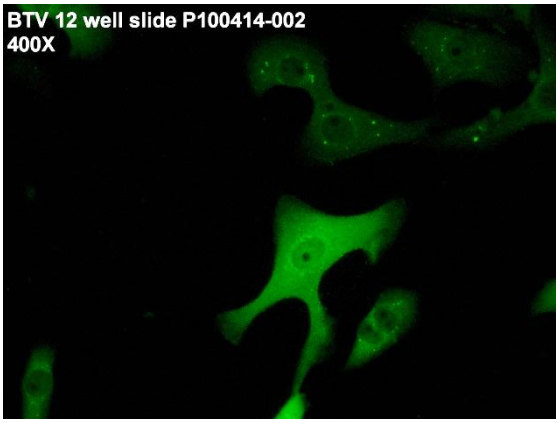
Certificate of Analysis

BLUETONGUE VIRUS (BTV)

IFA Substrate Slide



CATALOG NO.: SLD-IFA-BTV
SIZE: 12 Well
LOT: P100414-002
EXPIRATION: 10 February 2016

AGENT: Bluetongue Virus (BTV)
STRAIN: BT8

CELL CULTURE SUBSTRATE: Vero81

DESCRIPTION: Slides are virus-infected cell cultures grown on the surface of Teflon-masked slides. They are supplied fixed and unstained in moisture-free foil pouches. All wells contain both positive and negative cells. The positive cells usually total no more than 30% of the cells in the well so that good contrast may be seen.

QUALITY CONTROL METHOD: IFA using VMRD, Inc. BTV Positive Control (catalog no. PC-IFA-BTV), BTV Negative Control (catalog no. NC-IFA-BTV), and Anti-Bovine $IgG_{1,2}$ FITC Conjugate (catalog no. CJ-F-BOVG-AP-10ML).

Specific Reaction: 2-4+ fluorescence with trace background with the positive control and negative with no background with the negative control. 2-20 positive cells per high power field.

Other Comments: NA

PATTERN OF FLUORESCENCE: Multiple buckshot inclusions with dusty cytoplasmic fluorescence.

INTENDED USE: Generally used for Indirect FA to detect antibody to BTV but may also be used as a positive and negative control substrate slide for Direct FA conjugates when applicable. Screening dilution, 1/50.

STORAGE: Store sealed in foil pouch at -20°C. Avoid self-defrosting freezers.

REFERENCES: NA

FOR *IN VITRO* LABORATORY USE ONLY.

RECOMMENDED STAINING PROCEDURE FOR INDIRECT FA:

1. Warm slide to room temperature before removing from foil pouch.
2. Place 50 µl diluted serum on the designated wells. Dilute serum in serum diluting buffer, pH 7.2 (catalog no. FASDB-100ML).
3. Incubate slide in humid chamber at 37°C for 30 minutes.
4. Using a wash bottle, gently rinse slide briefly in FA rinse buffer, pH 9.0 (catalog no. FARB-4X) and then soak for 10 minutes in FA rinse buffer, pH 9.0.
5. Drain slide and dry around wells by pressing blotter (included in pouch) to front surface. Place 50 µl labeled anti-IgG or IgM on the wells.
6. Incubate as in step 3.
7. Rinse as in step 4.
8. Drain slide and dry back and edges with a paper towel. Do not allow stained surface to dry. Do not rinse with water.
9. Mount with mounting fluid [glycerol/FA rinse buffer, pH 9.0, (50/50)] (catalog no. FAMF-10ML) and view with good quality fluorescence microscope at 100X-250X. Confirmation may be made at 400X.

RECOMMENDED STAINING PROCEDURE FOR DIRECT FA:

1. Warm slide to room temperature before removing from foil pouch.
2. Place 50 µl of direct FA conjugate on the designated wells.
3. Incubate slide in humid chamber at 37°C for 30 minutes.
4. Using a wash bottle, gently rinse slide briefly in FA rinse buffer, pH 9.0 (catalog no. FARB-4X) and then soak for 10 minutes in FA rinse buffer, pH 9.0.
5. Drain slide and dry back and edges with a paper towel. Do not allow stained surface to dry. Do not rinse with water.
6. Mount with mounting fluid [glycerol/FA rinse buffer, pH 9.0, (50/50)] (catalog no. FAFM-10ML) and view with good quality fluorescence microscope at 100X-250X. Confirmation may be at 400X.

*SERUM DILUTING BUFFER (pH 7.2):**

- Na_2HPO_4 . . . . . 1.19 gm
- NaH_2PO_4 . . . . . 0.22 gm
- NaCl . . . . . 8.55 gm
- BSA . . . . . 10.0 gm
- DI/dH_2O . . . . . Q.S. to 1 liter

* This recipe makes 1 liter. If you need less, adjust recipe accordingly. Store at 2-7°C. Add 0.09% NaN_3 if diluted serum is not going to be used within one week.

4X FA RINSE BUFFER (pH 9.0):

- Na_2CO_3 . . . . . 11.4 gm
- $NaHCO_3$ . . . . . 33.6 gm
- NaCl . . . . . 8.5 gm
- DI/dH_2O . . . . . Q.S. to 1 liter

Final pH should be 9.0-9.5. This is a 4X concentrate and should be diluted 1/4 with DI/distilled water for use as a working buffer. Keep in a tightly stoppered container at room temperature. MOUNTING FLUID is made by mixing glycerol and FA rinse buffer, pH 9.0, in equal proportions.