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Certificate of Analysis

BLUETONGUE VIRUS (BTV)

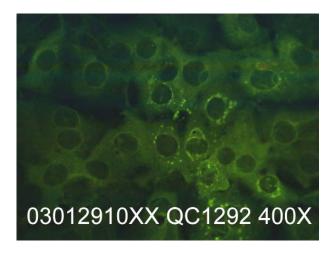
FA Substrate Slide

CATALOG NO.: 210-88-10-BT

SIZE: 10 well

LOT: 03012910XX-020605 **EXPIRATION:** 6 February 2005

AGENT: Bluetongue Virus (BTV)
Cell Culture Substrate: Vero
Virus Strain: BT8



QUALITY CONTROL METHOD: Indirect FA using VMRD BTV Positive Control (catalog no. 211-P-BTV), BTV Negative Control (catalog no. 211-N-BTV), and Anti-Bovine IgG_{1,2} FITC Polyclonal Conjugate (catalog no. 020-10).

Specific Reaction: 3+ positive on positive cells with some 4+ buckshot inclusions.

Other Reactions or Comments: No background; 0-10 positive cells per high power field.

PATTERN OF FLUORESCENCE: Multiple buckshot inclusions with dusty cytoplasmic fluorescence. 25-50% of cells are infected, showing fluorescence.

INTENDED USE: Useful in detecting viral antibody by IFA. Screening dilution, 1:50.

STABILITY: Foil-pouch sealed slides are stable for at least 6 months when stored below -20°C. Avoid self-defrosting freezers.

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.

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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. 210-93-SB).
- 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. 210-90-RB) 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 <u>back and edges</u> 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. 210-92-MF) 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. 210-90-RB) and then soak for 10 minutes in FA rinse buffer, pH 9.0.
- 5. Drain slide and dry <u>back and edges</u> 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. 210-92-MF) and view with good quality fluorescence microscope at 100X-250X. Confirmation may be at 400X.

SERUM DILUTING BUFFER (pH 7.2):*

-	Na ₂ HPO ₄	gm
-	NaH ₂ PO ₄	gm
-	NaCl	gm
-	BSA	gm
-	$\text{DI/dH}_2\text{O}\dots Q.S. \text{ to } 1$	liter

^{*} This recipe makes 1 liter. If you need less, adjust recipe accordingly. Store at 4°C. Add 0.09% NaN₃ 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	
-	NaCl	8.5 gm
-	DI/dH ₂ O	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.