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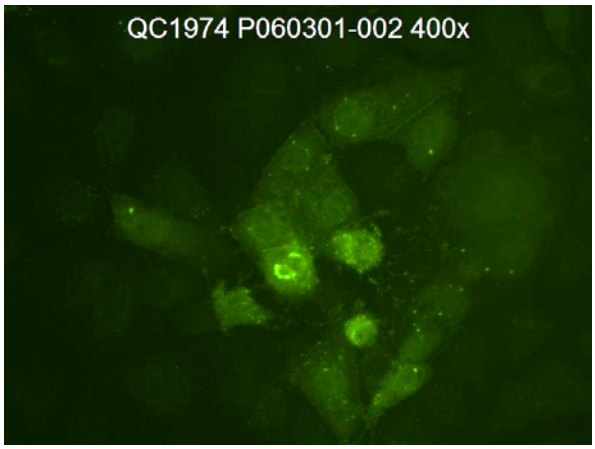
Certificate of Analysis

Bluetongue Virus (BTV)

Direct FA Conjugate



CATALOG NO.: 210-39-BT
VOLUME: 10 ml
LOT: P060301-002-030708
EXPIRATION: 7 March 2008

VIRUS: Bluetongue Virus (BTV)

DESCRIPTION: Anti-BTV monoclonal antibody conjugated to fluorescein isothiocyanate. Murine origin. Ready to use. Reacts with all U.S. and exotic serotypes. Does not react with EHDV.

QUALITY CONTROL METHOD: Direct FA using VMRD, Inc. BTV 10-well FA substrate slides (catalog no. 210-88-10-BT).

Specific Reaction: 2-3+ positive fluorescence on positive cells. Buckshot inclusions, diffuse cytoplasmic and nuclear fluorescence. Negative on negative cells.

Other Reactions or Comments: No background.

INTENDED USE: Useful for the detection of BTV in animal tissues or cell cultures.

STORAGE: This conjugate is provided in liquid form and should be stored at 4-8°C. DO NOT FREEZE! If conjugate becomes cloudy, it should be discarded. This conjugate contains 0.09% sodium azide as a preservative.

FOR IN VITRO LABORATORY USE ONLY.

RECOMMENDED STAINING PROCEDURE FOR DIRECT FA:

1. Air dry smears or tissue sections for at least 30 minutes at room temperature (do not dry cell cultures!).

2. Fix smears or tissue sections on slides for 20 minutes in acetone-methanol (75/25) at room temperature. Cell cultures should be rinsed with PBS and fixed in pure acetone for 10 minutes at room temperature. After fixation and before staining, slides should be dried for 10 minutes in a dry 37°C incubator.

3. Stain slides with 50-75 µl conjugate for 30 minutes at 37°C in humid chamber.

4. Gently rinse slides briefly in FA Rinse Buffer, pH 9.0 (VMRD catalog no. 210-90-RB) and then soak for 10 minutes in FA Rinse Buffer, pH 9.0.

5. Drain slides and dry back and edges with a paper towel. Do not allow stained surface to dry. Do not rinse with water.

6. Mount with FA Mounting Fluid [glycerol/FA rinse buffer, pH 9.0, (50/50)] (VMRD catalog no. 210-92-MF) and view with good quality fluorescence microscope at 100X-250X. Confirmation may be at 400X.

PHOSPHATE BUFFERED SALINE (PBS) SOLUTION (pH 7.2):

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

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.