

**VMRD**

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Certificate of Analysis

BLUETONGUE VIRUS (BTV)

IFA Positive Control Serum

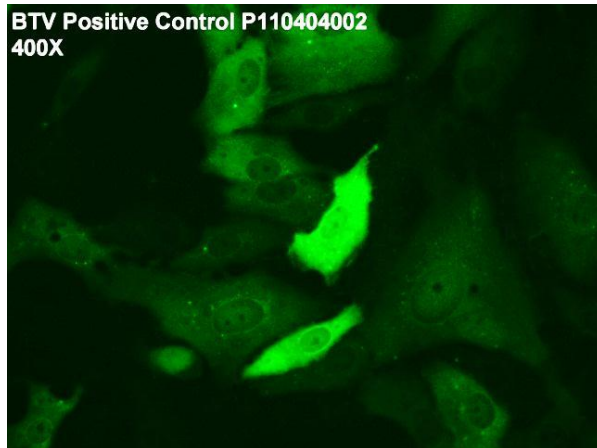
CATALOG NO.: PC-IFA-BTV

VOLUME: 1 ml

LOT: P110404-002

EXPIRATION: 23 November 2024

AGENT: Bluetongue Virus (BTV)

BTV Positive Control P110404002
400X**DESCRIPTION:** Bovine serum diluted in PBS, 1% BSA, 0.09% sodium azide.**QUALITY CONTROL METHOD:** IFA using VMRD, Inc. BTV 12-well slide (catalog no. SLD-IFA-BTV), BTV Negative Control (catalog no. NC-IFA-BTV), and Anti-Bovine IgG_{1,2} FITC Polyclonal Conjugate (catalog no. CJ-F-BOVG-AP-1ML or 10ML).**Specific Reaction:** 2–3+ signal at neat, no background. The endpoint titer was reached at a 1/16 dilution.**Other Comments:** NA**PATTERN OF FLUORESCENCE:** Multiple buckshot inclusions with dusty cytoplasmic fluorescence.**INTENDED USE:** As a positive control serum in detection of antibody to Bluetongue Virus by indirect FA technique. This serum should be used undiluted to demonstrate positive fluorescence.**STORAGE:** Store at 2–7°C. DO NOT FREEZE! If control becomes cloudy it should be discarded.**REFERENCES:** NA**FOR IN VITRO LABORATORY USE ONLY.**

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08 December 2020

RECOMMENDED STAINING PROCEDURE FOR INDIRECT FA:

1. Warm slide to room temperature before removing from foil pouch.
2. Place diluted serum on the designated wells. Dilute serum in serum diluting buffer, pH 7.2 (catalog no. FASDB-100ML) however if high background due to anti-bovine IgG activity is present it may be advisable to use SSDB-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 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 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. FAMF-10ML) and view with good quality fluorescence microscope at 100X-250X. Confirmation may be at 400X.

SERUM DILUTING BUFFER (pH 7.2):*

- Na₂HPO₄ 1.19 gm
- NaH₂PO₄ 0.22 gm
- NaCl 8.55 gm
- BSA 10.0 gm
- DI/dH₂O 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% NaN₃ if diluted serum is not going to be used within one week.

4X FA RINSE BUFFER (pH 9.0):

- Na₂CO₃ 11.4 gm
- NaHCO₃ 33.6 gm
- NaCl 8.5 gm
- DI/dH₂O 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.