CERTIFICATE OF ANALYSIS

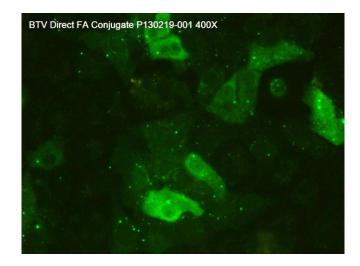
BLUETONGUE VIRUS (BTV)

Direct FA Conjugate

Catalog No.:	CJ-F-BTV-MAB-10ML
Volume:	10 ml
Lot:	P130219-001
Expiration:	06 March 2017
Agent:	Bluetongue Virus (BTV)
Strain:	NA

Description:

Anti-BTV monoclonal antibody conjugated to fluorescein isothiocyanate (FITC). Murine origin. Ready to use. Liquid.



Quality Control Method:

Direct FA using VMRD, Inc. BTV 12-well slide (catalog no. SLD-IFA-BTV).

Specific Reaction: 2-4+ fluorescence with no background.

Other Comments: Reacts with all U.S. and exotic serotypes. Does not react with Epizootic hemorrhagic disease virus (EHDV).

Pattern Of Fluorescence:

Multiple buckshot inclusions with dusty cytoplasmic fluorescence.

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 2-7°C. DO NOT FREEZE! It should also be stored in the original container and/or in the dark. If conjugate becomes cloudy it should be discarded. This conjugate contains 0.09% sodium azide as a preservative.

References: NA

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 $2-7\square$ 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
- 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.