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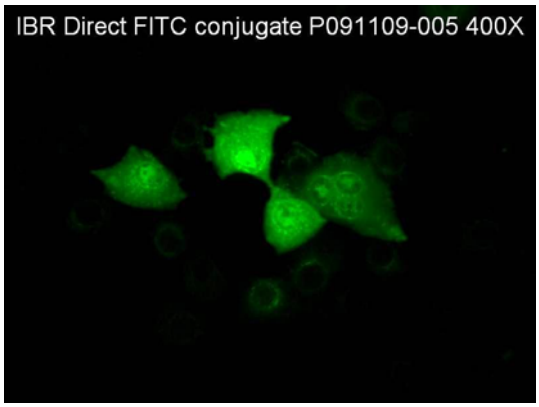
Certificate of Analysis

INFECTIOUS BOVINE RHINOTRACHEITIS VIRUS (IBR)

Direct FA Conjugate



CATALOG NO.: CJ-F-IBR-10ML
VOLUME: 10 ml
LOT: P091109-005
EXPIRATION: 26 September 2015

AGENT: Infectious Bovine Rhinotracheitis Virus/ Bovine Herpesvirus Type 1 (IBR/BHV-1)
STRAIN: NA

DESCRIPTION: Anti- IBR/BHV-1 polyclonal antiserum conjugated to fluorescein isothiocyanate (FITC). Caprine origin. Ready to use. Liquid.

QUALITY CONTROL METHOD: Direct FA on IBR/BHV-1-infected cell cultures using VMRD, Inc. control slides (catalog no. SLD-FAC-IBR) to detect binding.

Specific Reaction: 3-4+ fluorescence on positive well with trace background and negative on negative well.

Other Comments: The raw material (concentrated conjugate) has also been screened by Direct FA and was found to react with bovine adenovirus type 1 (BAV-1) at a signal of 2-3+ with 1+ background but does not react with other common bovine or caprine viruses.

PATTERN OF FLUORESCENCE: Primarily undifferentiated cytoplasmic with some nuclear fluorescence, especially in rounded cells and degenerating cells of plaques with acellular centers.

INTENDED USE: This reagent is useful for the detection of IBR/BHV-1 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

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 made 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. FA MOUNTING FLUID is made by mixing glycerol and FA Rinse Buffer, pH 9.0, in equal proportions.