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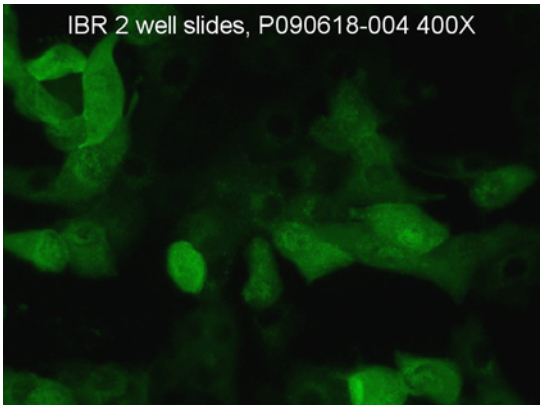
Certificate of Analysis

INFECTIOUS BOVINE RHINOTRACHEITIS VIRUS/BOVINE HERPESVIRUS TYPE 1(IBR/BHV-1)

FA Control Slide



CATALOG NO.: SLD-FAC-IBR
SIZE: 2 Well
LOT: P090618-004
EXPIRATION: 25 June 2011

AGENT: Infectious bovine rhinotracheitis virus/bovine herpesvirus type 1 (IBR/BHV-1)
STRAIN: Wyoming
CELL CULTURE SUBSTRATE: MDBK cells

DESCRIPTION: Slides are IBR-infected cell cultures grown on the surface of Teflon-masked slides. They are supplied fixed and unstained in moisture-free foil pouches. Each slide contains one positive and one negative cell culture well. The positive well contains 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.

QUALITY CONTROL METHOD: Direct FA using VMRD, Inc. IBR FA Conjugate (catalog no. 210-69-IBR or 210-69-IBR)

Specific Reaction: 1-4+ signal and trace background on positive well, no signal on negative well.
Other Comments: 3-100 infected cells and 1-5 plaques per high powered field.

PATTERN OF FLUORESCENCE: Undifferentiated cytoplasmic and nuclear fluorescence in single cells rounded by cytopathic effect (CPE).

INTENDED USE: For positive and negative control of direct or indirect FA tests for IBR/BHV-1.

STORAGE: Store sealed in foil pouch at -20°C. Avoid self-defrosting freezers.

REFERENCES: NA

FOR *IN VITRO* LABORATORY USE ONLY.

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 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. 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 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. 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_2HPO_4 . . . . . 1.19 gm
- NaH_2PO_4 . . . . . 0.22 gm
- NaCl . . . . . 8.55 gm
- BSA . . . . . 10.0 gm
- DI/dH_2O . . . . . 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% NaN_3 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
- $NaHCO_3$ . . . . . 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.