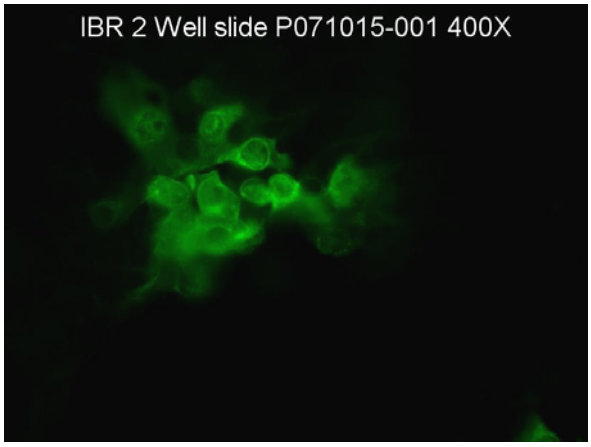
VMRD
PO Box 502, Pullman, WA 99163 USA

Telephone: + 1 (509) 334-5815
Fax: + 1 (509) 332-5356
E-mail: vmrd@vmrd.com
Web site: http://www.vmrd.com

Certificate of Analysis

Infectious Bovine Rhinotracheitis Virus/Bovine Herpesvirus-1 (IBR/BHV-1)

FA Control Slide



CATALOG NO.: SLD-FAC-IBR
SIZE: 2 well
LOT: P071015-001
EXPIRATION: 31 July 2011

AGENT: Infectious Bovine Rhinotracheitis Virus/ Bovine Herpesvirus-1 (IBR/BHV-1)
Cell Culture Substrate: MDBK Cells
Virus Strain: Wyoming

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

Specific Reaction: 3-4+ positive on positive well and negative on negative well with no background. 0 to 5 infected cells or plaques per high power field.

Other Reactions or Comments: NA

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

INTENDED USE: For positive and negative control of direct or indirect viral FA tests.

STABILITY: Foil-pouch sealed slides are stored below -20°C. Avoid self-defrosting freezers.

DESCRIPTION: Slides are virus-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.

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 4°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.