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# **Certificate of Analysis**

## **CANINE HERPESVIRUS**

FA Substrate Slide

**CATALOG NO.:** 210-88-10-CHV

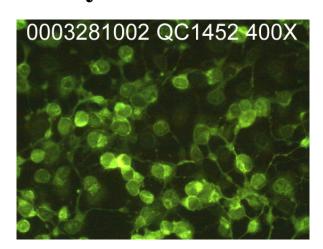
SIZE: 10 well

**LOT:** 0003281002-081405 **EXPIRATION:** 14 August 2005

**AGENT:** Canine Herpesvirus (CHV) **Cell Culture Substrate:** NBLDK cells

Virus Strain: Field isolate, Marshall

strain



**QUALITY CONTROL METHOD:** Indirect FA using VMRD CHV Positive Control (catalog no. 211-P-CHV); anti-Canine IgG FITC Conjugate (catalog no. 035-10).

**Specific Reaction:** 2-4+ positive fluorescence.

**Other Reactions or Comments:** Trace background. 2-4 plaques per low-power field; cell density is

higher than previous lot.

**PATTERN OF FLUORESCENCE:** Cytoplasmic and nuclear fluorescence in rounded cells with occasional small syncytia.

**INTENDED USE:** Useful in detecting viral antibody by IFA. May be used to differentiate antibody class (IgG or IgM) with suitable quality fluoresceinated second antibody conjugate.

**STABILITY:** Foil-pouch sealed slides are stable for at least 6 months when 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. All wells contain 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.

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#### 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). [If background is a problem, particularly at low dilutions, use of 10% adult bovine serum diluting buffer is preferable (catalog no. 210-94-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 <u>back and edges</u> 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 <u>back and edges</u> 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 <sub>2</sub> HPO <sub>4</sub>	1
-	NaH <sub>2</sub> PO <sub>4</sub>	1
-	NaCl	1
-	BSA	n
-	DI/dH <sub>2</sub> O	r

<sup>\*</sup> This recipe makes 1 liter. If you need less, adjust recipe accordingly. Store at 4°C. Add 0.09% NaN<sub>3</sub> if diluted serum is not going to be used within one week.

### 4X FA RINSE BUFFER (pH 9.0):

-	Na <sub>2</sub> CO <sub>3</sub>	
-	NaHCO <sub>3</sub>	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.