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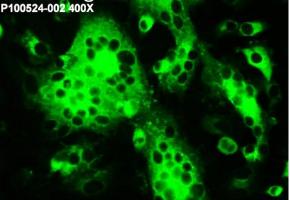
Certificate of Analysis

CANINE CORONAVIRUS (CCV)

Anti-Viral Antiserum CATALOG NO.: PAB-CCV VOLUME: 2 ml LOT: P100524-002 EXPIRATION: 13 March 2016

AGENT: Canine Coronavirus (CCV)

CCV Antiserum @ 1/400 P100524-002 400X



DESCRIPTION: CCV polyclonal antiserum. Liquid. Porcine origin.

QUALITY CONTROL METHOD: Indirect FA using VMRD Inc. CCV 12-well slide (catalog no. SLD-IFA-CCV) and Anti-Porcine IgG FITC Conjugate (catalog no. CJ-F-PORG-AP-1ML or 10ML). Specific Reaction: 3-4+ fluorescence at 1/400 with no background and an endpoint titer of 1/6400.
Other Comments: The Antiserum has also been screened by indirect FA and has been found to react with canine adenovirus type 2 (CAV-2) trace to 2+ at 1/100 with an endpoint of 1/200, canine

trace to 2+ at 1/100 with an endpoint of 1/200, canine herpesvirus type 1 (CHV-1) trace to 2+ at 1/100, this is the endpoint, canine parvovirus (CPV) with trace to 2+ at 1/100 with an endpoint of 1/200, but does not react with canine adenovirus type 1 (CAV-1), canine distemper virus (CDV), or canine parainfluenza virus type 2 (CPI-2).

PATTERN OF FLUORESCENCE: Large fluorescent syncytia with some single cells in between.

INTENDED USE: Useful for IFA. Not suitable for cell culture serum neutralization because it contains 0.09% sodium azide as a preservative.

STORAGE: This antiserum is provided in liquid form and should be stored at 2-7°C. DO NOT FREEZE! If antiserum becomes cloudy, it should be discarded. This antiserum contains 0.09% sodium azide as a preservative.

REFERENCES: NA

FOR IN VITRO LABORATORY USE ONLY.

WARRANTY: *VMRD, Inc.* warrants that this product is as described in the quantity and contents stated on the label at the time of delivery to the customer. NO OTHER WARRANTIES, EXPRESS OR IMPLIED, ARE MADE BEYOND THE LABEL DESCRIPTION, INCLUDING WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR USE. Remedy is limited to replacement of the product or refund of the purchase price. *VMRD, Inc.* is not liable for property damage, personal injury, or economic loss caused by the product. The information listed in this information sheet is provided for reference only, and should not be substituted for the user's own incoming material quality control.

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RECOMMENDED STAINING PROCEDURE FOR INDIRECT FA:

- 1. Warm slide to room temperature before removing from foil pouch.
- Place 50 μl 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 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. 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 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. FARB-4X) 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. FAMF-10ML) and view with good quality fluorescence microscope at 100X-250X. Confirmation may be at 400X.

SERUM DILUTING BUFFER (pH 7.2):*

-	Na ₂ HPO ₄
-	NaH ₂ PO ₄ 0.22 gm
	NaCl
-	3SA10.0 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% NaN₃ if diluted serum is not going to be used within one week.

4X FA RINSE BUFFER (pH 9.0):

-	Na ₂ CO ₃	. 11.4 gm
-	NaHCO ₃	.33.6 gm
-	NaCl	8.5 gm
-	DI/dH ₂ OQ.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.