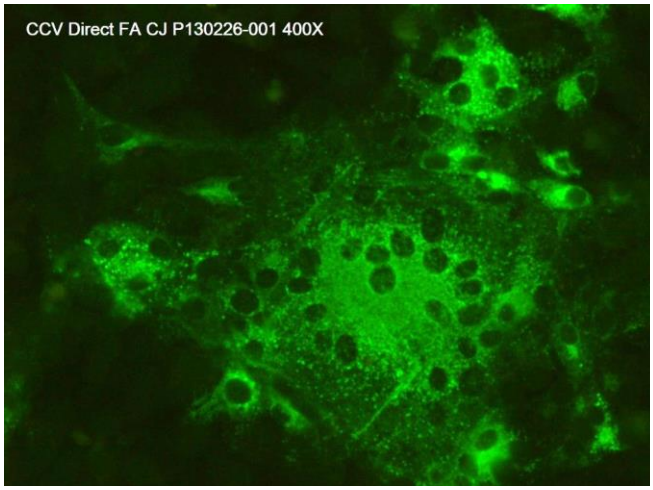
Veterinary Medical
Research & Development

CERTIFICATE OF ANALYSIS

CANINE CORONAVIRUS (CCV)

Direct FA Conjugate

Catalog No.:	CJ-F-CCV-10ML
Volume:	10 ml
Lot:	P130226-001
Expiration:	17 February 2021
Agent:	Transmissible Gastroenteritis virus (TGEV)
Strain:	NA



Description:

TGEV polyclonal antiserum conjugated to fluorescein isothiocyanate (FITC). Porcine origin. Ready to use. Liquid.

Quality Control Method:

Direct FA using VMRD Inc. CCV 2-well slide (catalog no SLD-FAC-CCV).

Specific Reaction: 2-4+ signal no background on the positive well, and negative no background on the negative well.

Other Comments: The raw material has also been screened by indirect FA and has been found to react with porcine cytomegalovirus (PCMV) at 2+ but does not react with porcine adenovirus (PAV), porcine circovirus type 1 and 2 (PCV-1 and 2), porcine hemagglutinating encephalomyelitis virus (PHEV), porcine parvovirus (PPV), porcine reproductive and respiratory syndrome virus (PRRSV), and vesicular stomatitis virus Indiana or New Jersey strains (VSV), *Anaplasma phagocytophila*, canine adenovirus type 1 and 2 (CAV-1 and 2), *Brucella canis*, canine distemper virus (CDV), canine herpesvirus type 1 (CHV-1), canine parainfluenza virus type 2 (CPI-2), canine parvovirus (CPV), *Ehrlichia canis*, *Borrelia burgdorferi* (Lyme disease), *Leishmania infantum*, *Neospora caninum* (canine origin), *Rickettsia rickettsii* (RMSF), and *Toxoplasma gondii*.

Pattern Of Fluorescence:

Large fluorescent syncytia with some single cells in between.

Intended Use:

Detects CCV in gut sections or smears, and in cell cultures for identification of isolates.

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

PO Box 502
Pullman, WA 99163 USA

P: 509.334.5815
F: 509.332.5356

techserve@vmrd.com
www.vmrd.com

Recommended Staining Procedure for Indirect FA:

1. Warm slide to room temperature before removing from foil pouch.
2. Place 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 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. 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 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 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. FAMF-10ML) 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.