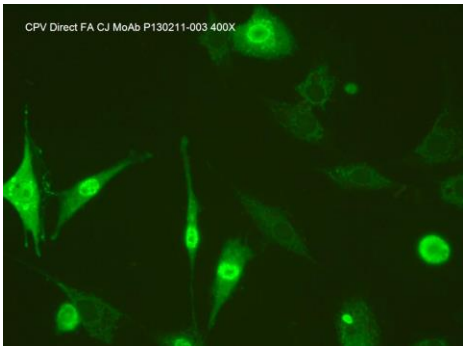
Veterinary Medical
Research & Development

CERTIFICATE OF ANALYSIS

Canine Parvovirus (CPV)

Direct FA Conjugate

Catalog No.:	CJ-F-CPV-MAB-10ML
Volume:	10 ml
Lot:	P130211-003
Expiration:	11 April 2021
Agent:	Canine Parvovirus (CPV)
Strain:	NA



Description:

Monoclonal antibody conjugated to fluorescein isothiocyanate (FITC). Murine origin. Ready to use. Liquid.

Quality Control Method:

Direct FA using CPV 2-well slide (catalog no. SLD-FAC-CPV).

Specific Reaction: 2-4+ fluorescence on the positive well, negative on the negative well. Trace background on both wells.

Other Comments: The raw material has also been screened by Indirect FA and does not react with *Anaplasma phagocytophila*, canine adenovirus type 1 and 2 (CAV 1 and 2), canine coronavirus (CCV), *Brucella canis* (canine brucellosis), canine distemper virus (CDV), canine herpesvirus type 1 (CHV), canine parainfluenza virus type 2 (CPI-2), *Ehrlichia canis*, *Borrelia burgdorferi* (Lyme disease), *Leishmania infantum*, *Neospora caninum* (canine origin), *Rickettsia rickettsii* (RMSF), *Toxoplasma gondii*.

Pattern Of Fluorescence:

Single cells with cytoplasmic and nuclear fluorescence.

Intended Use:

This conjugate may be used to stain CPV either in gut tissues or cell cultures. Especially useful in titration or isolating CPV in cell cultures.

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:

Parish, C.R., *et al.* Antigenic Relationships Between Canine Parvovirus Type 2 Feline Panleukopenia Virus and Mink Enteritis Virus Using Conventional Antisera and Monoclonal Antibodies. *Archives of Virology* 72, 267-278(1982).

Parrish, C.R. and Carmichael, L. E. Antigenic Structure and Variation of Canine Parvovirus Type-2, Feline Panleukopenia Virus and Mink Enteritis Virus. *Virology* 129, 401-414 (1983).

Strassheim, M.L., *et al.* Two dominant neutralizing antigenic determinants of canine parvovirus are found on the threefold spike of the virus capsid. *Virology* 198(1):175-184 (Jan. 1994).

Wikoff, W.R., *et al.* The structure of a neutralized virus: Canine parvovirus complexed with neutralizing antibody fragment. *Structure* 2(7):595-607 (July 1994).

Ramos-Vara, J.A., and M.E. Beissenherz. Optimization of immunohistochemical methods using two different antigen retrieval methods on formalin-fixed, paraffin-embedded tissues: Experience with 63 markers. *J. Vet. Diagn. Invest.* 12(4):307-311 (July 2000).

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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.