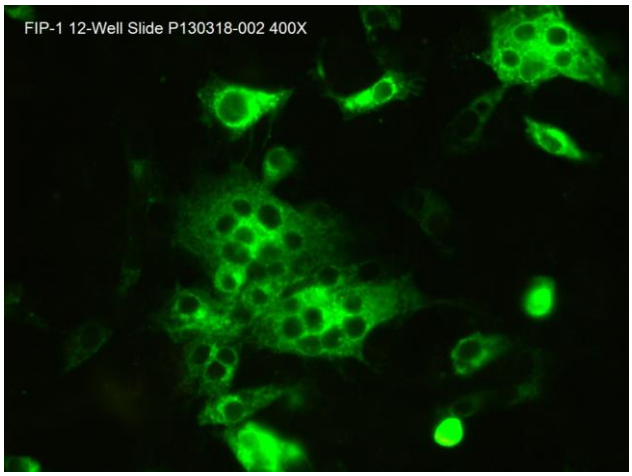
Veterinary Medical
Research & Development

CERTIFICATE OF ANALYSIS

FELINE INFECTIOUS PERITONITIS TYPE 1 (FIP-1)

IFA Substrate Slide

Catalog No.:	SLD-IFA-FIP1
Size:	12 Well
Well Capacity:	50 µl
Lot:	P130318-002
Expiration:	18 April 2017
Agent:	Feline Coronavirus Type 1 (FIP-1)
Strain:	TN-406
Cell Culture Substrate:	CrFK (TENN)



Description:

Wells contain virus-infected cell cultures grown on the surface of Teflon-masked slide. Slides 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.

Quality Control Method:

Indirect FA using FIP-1 positive control (catalog no. PC-IFA-FIP1), FIP-1 negative control (catalog no NC-IFA-FIP1), and Anti-Feline IgG FITC AP conjugate (catalog no. CJ-F-FELG-AP-1ML or 10ML).

Specific Reaction: 4+ Fluorescence with the positive control, no background and negative with the negative control, trace background. There are 0 to 50 positive cells and or syncytia per high-powered field.

Other Comments: NA

Pattern Of Fluorescence:

Multiple syncytia, plaques and some individual cells with granular cytoplasmic fluorescence.

Interpretation Of Results:

For cats expressing clinical signs consistent with FIP, we recommend testing sera at a screening dilution of 1/6400. Screening of healthy cats should be at 1/400. For the 1/6400 screening dilution, we recommend using a single-step dilution of 1 µl of test serum in 6.4 ml of diluent or a 2-step 1/64 and 1/100 for the most consistent interlaboratory results. Making serial dilutions of this magnitude may compound errors of various kinds. Endpoint titers may be determined from four-fold dilutions of the 1/6400 or 1/400 screening dilutions. Sera diluted in PBS should be assayed within 1 hour of making the dilution.

Intended Use:

Generally used for Indirect FA to detect antibody to FIP1 but may also be used as a positive and negative control substrate slide for Direct FA conjugates when applicable.

Storage:

Store sealed in foil pouch at -20°C. Avoid self-defrosting freezers.

References: NA

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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.