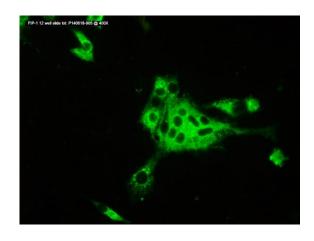


CERTIFICATE OF ANALYSIS

Feline Infectious Peritonitis Virus Type 1 (FIP-1)

FA Substrate Slide

Catalog No.:	SLD-IFA-FIP1
Size:	12 Well
Well Capacity:	50 μΙ
Lot:	P140618-005
Expiration:	04 August 2018
Agent:	Feline Coronavirus
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), negative control (catalog no NC-IFA-FIP1), and anti-feline IgG FITC affinity purified conjugate (catalog no. CJ-F-FELG-AP-1ML or 10ML).

Specific Reaction: 3-4+ fluorescence with the positive control at neat, no background. The negative

control was trace with no background. There are 0 to 50 infected cells and or syncytia

per high-power 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 μ l of test serum in 6.4 ml of diluent or a 2-step 1/64 and 1/100 for the most consistent inter laboratory 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 FIP-1 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

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 ₂ HPO ₄	1.19 gm
-	NaH ₂ PO ₄	0.22 gm
-	NaCl	8.55 gm
-	BSA	10.0 gm
_	DI/dH ₂ O	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
-	NaCl8.5 gm
_	DI/dH₂OQ.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.