Cat feline sarcoma oncogene (FES) ELISA kit

Catalog No. CSB-EL008597CA

(96 T)

- This immunoassay kit allows for the in vitro quantitative determination of **cat FES** concentrations in **serum, plasma**，t**issue homogenates and cell lysates.**
- **Expiration date** **six** months from the date of manufacture
- **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

PRINCIPLE OF THE ASSAY

The microtiter plate provided in this kit has been pre-coated with an antibody specific to FES. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated antibody preparation specific for FES and Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Then a TMB (3,3',5,5' tetramethyl-benzidine) substrate solution is added to each well. Only those wells that contain FES, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of FES in the samples is then determined by comparing the O.D. of the samples to the standard curve.

DETECTION RANGE

The standard curve concentrations used for the ELISA's are ranging from 15.6 pg/ml -1000 pg/ml.

SPECIFICITY

This assay recognizes cat FES. No significant cross-reactivity or interference was observed.

SENSITIVITY

The minimum detectable dose of cat FES is typically less than 3.9 pg/ml.

The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest protein concentration that could be differentiated from zero.

MATERIALS PROVIDED AND STORAGE

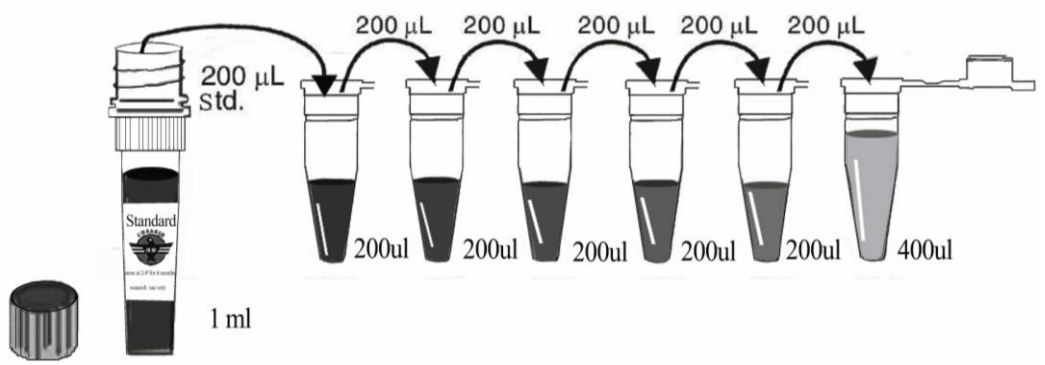
	Reagent	Quantity	Storage
Unopened kit	Store at 2 - 8° C. Do not use beyond kit expiration date.		
Opened kit	Coated plate	1	May be stored for up to 1 month at 2 - 8° C. Try to keep it in a sealed aluminum foil bag,and avoid the damp
	Standard	2	May be stored for up to 1 month at 2 - 8° C. If don't make recent use, better keep it store at -20℃.
	Biotin-antibody	1 x120ul	
	HRP-avidin	1 x120ul	
	Sample Diluent	1 x 20 ml	May be stored for up to 1 month at 2 - 8° C.
	Biotin-antibody Diluent	1 x 10 ml	
	HRP-avidin Diluent	1 x 10 ml	
	Wash Buffer	1 x 20 ml **(25×concentrate)**	
	TMB Substrate	1 x 10 ml	
	Stop Solution	1 x 10 ml	

REAGENT PREPARATION

Bring all reagents to room temperature before use for 30min..

1. **Wash Buffer** If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to prepare 500 ml of Wash Buffer.
2. **Biotin-antibody** Centrifuge the vial before opening. Dilute to the working concentration using **Biotin-antibody Diluent**(1:100), respectively. The suggested 100-fold dilution can be achieved by adding 10 uL **Biotin-antibody** to 990uL of **Biotin-antibody Diluent** for 1ml working solution.
3. **HRP-avidin** Centrifuge the vial before opening. Dilute to the working concentration using **HRP-avidin Diluent**(1:100), respectively. The suggested 100-fold dilution can be achieved by adding 10 uL **HRP-avidin** to 990uL of **HRP-avidin Diluent** for 1ml working solution.

4. **Standard** Centrifuge the standard vial at 6000-10000rpm for 30s. Reconstitute the **Standard** with 1.0 ml of **Sample Diluent**. This reconstitution produces a stock solution of 1000 pg/ml. Allow the standard to sit for a minimum of 15 minutes with gentle and uniform agitation by pipette with 1ml measuring range prior to making serial dilutions. The undiluted standard serves as the high standard (1000 pg/ml). The **Sample Diluent** serves as the zero standard (0 pg/ml). Prepare fresh for each assay. Use within 4 hours and discard after use.



Standard	S7	S6	S5	S4	S3	S2	S1
Concentration (pg/ml)	1000	500	250	125	62.5	31.2	15.6

Precaution: The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- An incubator which can provide stable incubation conditions up to 37°C±0.5°C.

SAMPLE COLLECTION AND STORAGE

- **Serum** Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 g. Remove serum and assay immediately or aliquot and store samples at -20°C. Centrifuge the sample again after thawing before the assay. Avoid repeated freeze-thaw cycles.
- **Plasma** Collect plasma using citrate, EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 g within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C. Centrifuge the sample again after thawing before the assay. Avoid repeated freeze-thaw cycles.

- **Tissue Homogenates** 100mg tissue was rinsed with 1X PBS, homogenized in 1 mL of 1X PBS and stored overnight at -20° C. After two freeze-thaw cycles were performed to break the cell membranes, the homogenates were centrifuged for 5 minutes at 5000 x g, 2 - 8°C. The supernate was assayed and removed immediately. Alternatively, aliquot and store samples at -20°C or -80℃. Centrifuge the sample again after thawing before the assay. Avoid repeated freeze-thaw cycles。
- **Cell lysates** Remove media and rinse cells once with ice-cold PBS (PH7.2-7.4). Scrape cells off the plate and transfer to an appropriate tube. Dilute cell suspension with 1xPBS (PH7.2-7.4), until cell concentration reached 100 million/ml. Then store overnight at -20° C. After two freeze-thaw cycles to break up the cell membranes, the cell lysates were centrifuged for 5 minutes at 5000 g, 2 - 8°C. Collect the supernatant. Cell lysates should be assayed immediately or aliquotted and stored at -20° C. Centrifuge the sample again after thawing before the assay. Avoid repeated freeze-thaw cycles.

Note: Grossly hemolyzed samples are not suitable for use in this assay.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended

that all samples, standards, and controls be assayed in duplicate. All the reagents should be added directly to the liquid level in the well. The pipette should avoid contacting the inner wall of the well.

1. Add 100μl of Standard, Blank, or Sample per well. Cover with the adhesive strip. Incubate for 2 hours at 37°C.
2. Remove the liquid of each well, don't wash.
3. Add 100μl of **Biotin-antibody** working solution to each well. Incubate for 1 hour at 37°C. **Biotin-antibody** working solution may appear cloudy. Warm up to room temperature and mix gently until solution appears uniform.
4. Aspirate each well and wash, repeating the process three times for a total of three washes. Wash: Fill each well with Wash Buffer (200μl) and let it stand for 2 minutes, then remove the liquid by flicking the plate over a sink. The remaining drops are removed by patting the plate on a paper towel. Complete removal of liquid at each step is essential to good performance.
5. Add 100μl of **HRP-avidin** working solution to each well. Cover the microtiter plate with a new adhesive strip. Incubate for 1 hour at 37°C.
6. Repeat the aspiration and wash five times as step 4.
7. Add 90μl of **TMB Substrate** to each well. Incubate for 15-30 minutes at 37°C. Keeping the plate away from drafts and other

temperature fluctuations in the dark.

8. Add 50µl of **Stop Solution** to each well when the first four wells containing the highest concentration of standards develop obvious blue color. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

Using the professional soft "Curve Exert 1.3" to make a standard curve is recommended, which can be downloaded from our web.

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the x-axis against the concentration on the y-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the FES concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples

have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

LIMITATIONS OF THE PROCEDURE

- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- It is important that the Standard Diluent selected for the standard curve be consistent with the samples being assayed.
- If samples generate values higher than the highest standard, dilute the samples with the appropriate Standard Diluent and repeat the assay.
- Any variation in Standard Diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- Centrifuge vials before opening to collect contents.
- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate Solution should remain colorless or light blue until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless or light blue to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

PRECISION

Intra-assay Precision (Precision within an assay)

One sample whose concentration between the highest and the second standard were tested twenty times on one plate to assess.

CV%<8%

Inter-assay Precision (Precision between assays)

One sample whose concentration between the highest and the second standard were tested in thirty-five separate assays to assess

CV%<10%

PLATE LAYOUT

Use this plate layout as a record of standards and samples assayed.

1 2 3 4 5 6 7 8 9 10 11 12
A B C D E F G H