

ANITIA Feline IgE I ANITIA Feline IgE II

IVD **REF** PFIE0112/PFIF0112

Immunoblot assay for the semi-quantitative detection of allergen-specific IgE antibodies in feline serum

[Intended use]

ANITIA Feline IgE I, ANITIA Feline IgE II are an *in vitro* diagnostic test for semi-quantitative determination of allergen-specific IgE concentration in feline serum using immunoblotting technique.

[Summary and explanation of the test]

Atopic allergy is a hypersensitive immunological condition mediated by immunoglobulin E (IgE) antibodies. Lymphocyte B cells stimulated by a specific allergen produce IgE antibodies to the antigen. The IgE antibodies bind to the receptors on the surface of mast cells or basophilic leukocytes through Fc region. Subsequent binding of the allergen to cell-bound specific IgE triggers cell degranulation and the release of vasoactive amines causing smooth muscle contraction, itch, swelling and transmucosal leakage of extracellular fluids. The most common clinical manifestations of this biological process are hay fever, asthma, dermatitis, hives, and anaphylactic shock. The evaluation of IgE level in the patient's serum for various allergens is valuable in the diagnosis and treatment of atopic allergy.

[Test principle]

ANITIA Feline IgE I, ANITIA Feline IgE II, a multiplex allergy diagnostic kit, based on the principle of an enzyme immunoassay includes nitrocellulose membranes which have various allergens adsorbed at regular interval lines, which makes it possible to test dozens of specific allergens in one test. **ANITIA Feline IgE I, ANITIA Feline IgE II** can test variety of allergens in one test by employing a new technique to arrange membranes in parallel compared to other products which have just one lane membrane. If allergen-specific IgE antibodies bind to the antigens, they are immobilized on the membrane after the washing step. The immobilized IgE antibodies bind to biotin-coupled anti-feline IgE antibodies, and then the biotin is captured by a streptavidin conjugated with alkaline phosphatase. The color is developed after adding the substrate in the last incubation step by the enzyme and the intensity of color is analyzed using a color-measuring device.

[Provided reagents] 1 Kit

No.	Name	Symbol	Quantity
1	Allergen panel	Allergen Panel	10 Tests x 2 E.A.
2	Sample diluent	Sample DiL	10 mL x 1 E.A.
3	Antibody solution	Antibody SOLN	10 mL x 1 E.A.
4	Enzyme solution	Enzyme SOLN	10 mL x 1 E.A.
5	Substrate solution	Substrate SOLN	10 mL x 1 E.A.
6	Washing solution 20x	Wash SOLN 20x	20 mL x 1 E.A.

※Additionally necessary equipment

- Manual method: Q-Smart (V) (Optical measuring device), Pipette, Suction kit and Orbital shaker or its equivalent
- Semi-automatic method: Q-Smart (V) (Optical measuring device), Q-Processor (V) (Automatic device for incubation and washing), Q-STATION ELITE (V) (Automatic device for measuring)
- Fully-automatic method: Q-STATION ELITE (V) (Automatic device for dispensing of samples and reagents, incubation, washing, drying and measuring)

[Appearance]

- Allergen panel: A light purple (ANITIA Feline IgE I) or purple (ANITIA Feline IgE II) plastic panel with two white membranes are attached in parallel
- Sample diluent: Blue violet liquid
- Antibody solution: Yellow liquid
- Enzyme solution: Colorless liquid
- Substrate solution: Light-yellow liquid
- Washing solution 20x: Colorless liquid

[Test procedure]

1. Before the test

All the reagents should be equilibrated to room temperature (15~25℃) before 30 minutes of use and mixed well immediately before use. Open the packaging of allergen panel after equilibration to room temperature.

NOTE Allergen panels not used for the test should be immediately put into a sealed aluminum pouch and keep refrigerated.

2. Preparation of reagents and specimens

(1) Preparation of washing solution (1x)

Dilute the washing solution 20x 20 times with deionized water before test.

Ex) 19 mL deionized water + 1 mL washing solution 20x = 20 mL washing solution 1x

(2) Preparation of specimens

Serum is used in the test. Remove blood cells or any solid matters by centrifugation before test. Hemolyzed or contaminated samples may cause incorrect results. Store serum samples at 2~8℃ (refrigerating temperature) when they are used for a short period (within 1 week) and at -20℃ or below (freezing temperature) for longer use. Repeated freezing and thawing of serum samples should be avoided.

3. Test methods

Manual method

- Wet the test membrane completely with 300 µL of diluted washing solution (1x) by shaking for 5 minutes and remove the washing solution (1x) (100 rpm is recommended at orbital shaker).
- Fill the allergen panels with 250 µL of sample diluent.
- Add 50 µL of patient sample and incubate with shaking at room temperature for 45 minutes.
- Remove the sample from the panel and wash the membrane twice with the diluted washing solution. At every washing step, add 300 µL of diluted washing solution, incubate with shaking for 5 minutes at room temperature and empty the panel. Solution should not remain in the panel.
- Add 250 µL of antibody solution to the panel and incubate with shaking at room temperature for 45 minutes.
- Rinse off the membrane as the method 4).
- Fill the panel with 250 µL of enzyme solution and incubate with shaking for 30 minutes at room temperature.
- Rinse off the membrane as the method 4).
- Add 250 µL of the substrate solution and incubate with shaking at room temperature in a dark room for 20 minutes.
- Remove the substrate solution from the panel, rinse the membrane once with 250 µL of deionized water.
- Dry the membrane in the air or with a dryer (Please make sure that the membrane is dried completely.)
- Insert the panel into Q-Smart (V) or Q-STATION ELITE (V) and evaluate the results. Please refer to each user manual for details.

Semi-automatic method

- Insert the Q-Processor (V) with allergen panels and reagents.
- Refer to the user manual of Q-Processor (V) and follow the directions.
- Dry the membranes as the manual method and evaluate the results using Q-Smart (V) or Q-STATION ELITE (V). Please refer to each user manual for details.

Fully-automatic method

- Insert the Q-STATION ELITE (V) with allergen panels and reagents.
- Refer to the user manual of Q-STATION ELITE (V) and follow the directions. Q-STATION ELITE (V) will automatically perform the entire procedures of dispensing patient samples and reagents, incubation, washing, drying and measurement.

4. Evaluation and interpretation

The amount of allergen-specific IgE antibodies is quantitatively analyzed as AU/mL via Q-Smart (V) and Q-STATION ELITE (V). The class is determined using the below table.

Allergen-specific IgE		
AU/mL	Class	Amount of allergen-specific IgE
≤ 0.34	0	Not found
0.35~3.49	1	Weak
3.50~49.99	2	Moderate
≥ 50.00	3	Strong

5. Quality control

The control line (C1) should develop stronger than the standard line (S3). If the control line (C1) does not develop stronger than the standard line (S3), it cannot be read normally by optical measuring devices. In that case, re-test is recommended.

5. Performances

1) Analytical sensitivity

- Limit of Detection (LoD): 0.32 AU/mL
- Cut-off value: 0.35 AU/mL

2) Analytical specificity

There is no detectable interference and cross-reactivity with the following concentration.

Interference and cross-reactivity substances	Concentration
Cholesterol	10 mg/mL
Glucose	6 mg/mL
Biotin	0.03 mg/mL
Bilirubin (unconjugated)	0.005 mg/mL
Cat IgG	25.0 mg/mL
Cat IgM	3.0 mg/mL

3) Precision (CV): Less than 20% for both intra-assay variation and inter-assay variation

[Precautions for use]

- For *in vitro* diagnostic and veterinary (professional) use only.
- ANITIA Feline IgE I, ANITIA Feline IgE II** can be used for helping the clinical diagnosis and definitive clinical diagnosis or dosage regimens for immunotherapy should be decided by the veterinarian after all clinical and laboratory findings are evaluated.
- It is possible that discrepancies may occur between the results from **ANITIA Feline IgE I, ANITIA Feline IgE II** and those from *in vivo* tests and/or from other *in vitro* tests since there are no national or international standards and the allergen extracts may be different among tests.
- False positive test results could be produced by cross-reactivity with other allergens.
- This product must be used by the trained expert according to the procedures in the user manual.
- Do not smoke, eat or drink in areas where test samples or products are used.
- All test samples may have a potential to include unknown infectious materials. When handling the samples, wear disposable gloves and wash your hands after test.
- Do not use samples that have been hemolyzed or contain lipid since they can affect the test results.

- 9) Do not place needles, knives or any other objects that can cause injury while handling feline samples and products to avoid safety accidents.
- 10) All used feline samples and product components should be regarded as the bio-hazards. It should be disposed according to the relevant guidelines.
- 11) Do not use products after the expiration date.
- 12) Do not mix and use the solutions from different Lot No.
- 13) Keep the products refrigerated at 2~8°C and be careful not to be frozen.
- 14) The allergen panels are packaged with a desiccant. The stability can be decreased if the allergen panel is exposed to moisture. Unused allergen panels should be tightly sealed and keep refrigerated after unpacking.
- 15) Some reagents included in the kit contain sodium azide as a preservative. Sodium azide has been reported to react with lead or copper which forms potentially explosive metal azide. Therefore, always flush with excessive volume of water to prevent metal azide in plumbing systems.
- 16) Do not expose substrate solution to light when using or storing the solution.
- 17) Substrate solution may contain black precipitates. This is not an abnormal phenomenon and has no effect on test results.
- 18) Be careful to avoid the forming of bubbles during the test. Especially when the automatic devices are used, bubbles should be removed before starting the test since they can affect the test volume.
- 19) If the temperature in the laboratory is too low or the membrane is not completely dried after the final reaction, the test results analyzed by measuring devices may be incorrect.

[Package unit] 1 Kit (for 20 Tests)

[Storage condition] Store at 2~8°C

[Use by] 24 months after the manufacturing date (3 months after open)

[Understanding of symbol marks]

LOT Lot No. Store at 2~8°C

IVD In vitro diagnostic medical device

REF Catalog number

Manufacturer

Use by

Be cautious in use and consult instructions for use

Consult instructions for use

20 tests per kit

Manufacturer

ProteomeTech Inc.
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[Test panel]

1) ANITIA Feline IgE I

No. ⁽¹⁾	Allergens ⁽²⁾	Code	No. ⁽¹⁾	Allergens ⁽²⁾	Code
1	<i>Dermatophagoides pteronyssinus</i>	d1	31	Cockroach	i6
2	<i>Dermatophagoides farinae</i>	d2	32	Pork	f26
3	<i>Acarus siro</i>	d70	33	Beef	f27
4	<i>Tyrophagus putrescentiae</i>	d72	34	Duck meat	f581
5	Alder/ Birch	t2/ t3	35	Chicken	f83
6	Hazel	t4	36	Lamb meat	f88
7	Maple leaf sycamore	t11	37	Turkey meat	f284
8	Willow/ Cottonwood	t12/ t14	38	Red deer	f867
9	Oak	t7	39	Wheat	f4
10	White pine	t16	40	Corn	f8
11	Acacia	t19	41	Rice	f9
12	White ash	t15	42	Pea	f12
13	Common ragweed	w1	43	Soy bean	f14
14	Plantain	w9	44	Carrot	f31
15	Mugwort	w6	45	Potato	f35
16	Sheep's sorrel	w18	46	Sweet potato	f54
17	Bermuda grass	g2	47	Pumpkin	f225
18	Orchard grass / Timothy grass	g3/ g6	48	Tomato	f25
19	Ryegrass	g5	49	Apple	f49
20	Cultivated rye	g12	50	Yeast, baker's	f45
21	Dog dander	e5	51	Egg white	f1
22	Wool, sheep	e81	52	Egg yolk	f75
23	Feather mix	ex1	53	Milk	f2
24	<i>Penicillium notatum</i>	m1	54	Cheddar cheese / Gouda cheese	f81
25	<i>Cladosporium herbarum</i>	m2	55	Crab/ Shrimp	f23/ f24
26	<i>Aspergillus fumigatus</i>	m3	56	Codfish	f3

27	<i>Candida albicans</i>	m5	57	Tuna	f40
28	<i>Alternaria alternata</i>	m6	58	Salmon	f41
29	<i>Malassezia pachydermatis</i>	m227	59	Mackerel	f206
30	Flea	B22	60	Peanut	f13

2) ANITIA Feline IgE II

No. ⁽¹⁾	Allergens ⁽²⁾	Code	No. ⁽¹⁾	Allergens ⁽²⁾	Code
1	House dust	h1	31	Herring	f205
2	<i>Glycyphagus domesticus</i>	d73	32	Sardine	f308
3	<i>Blomia tropicalis</i>	d201	33	Anchovy	f313
4	Japanese hop	w22	34	Sea bass	f410
5	CCD (Cross-reactive Carbohydrate Determinant)	o214	35	Barley meal	f6
6	Peach	f95	36	Oat	f7
7	Sweet vernal grass	g1	37	Buckwheat	f11
8	Common reed	g7	38	Millet	f56
9	Bent grass	g9	39	Lentil	f235
10	Japanese cedar	t17	40	Sweet chestnut	f299
11	Ox-eye daisy	w7	41	Linseed (Flax seed)	f333
12	Dandelion	w8	42	Orange	f33
13	Russian thistle	w11	43	Strawberry	f44
14	Goldenrod	w12	44	Blueberry	f288
15	Common pigweed	w14	45	Kiwi	f84
16	Hevea latex	k82	46	Melon	f87
17	Bee venom	i1	47	Mango	f91
18	Fire ant	i70	48	Banana	f92
19	Mosquito	i71	49	Pear	f94
20	Cattle epithelium	e4	50	Pineapple	f210
21	Silkworm pupa	-	51	Plum	f255
22	α -lactalbumin	f76	52	Watermelon	f329
23	β -lactoglobulin	f77	53	Parsley	f86
24	Casein	f78	54	Cabbage	f216
25	Buttermilk	f805	55	Cucumber	f244
26	Gluten	f79	56	Broccoli	f260
27	Yeast, Brewer's	f450	57	Cauliflower	f291
28	Rabbit meat	f213	58	Radish	f310
29	Blue mussel/ Clam	f37/ f207	59	Paprika	f218
30	Trout	f204	60	Spinach	f214

(1) Refer to the picture below for the line numbers

(2) Extracted allergen from

