

**DRG<sup>®</sup> Feline Calici Virus Ab ELISA (EIA-2473)****Revised 1 Aug. 2008 (Vers. 3.0)****For Veterinary Use Only****INTRODUCTION**

Demonstration of serum antibodies is the most commonly used method for the diagnosis of Feline Calici Virus (FCV) infection or for monitoring the efficacy of vaccination. Feline Calici plays an important role in a complex disease of both wild and domestic cats ("sneezing disease").

Important in the diagnosis of Feline Calici infection:

- Clinical history
- Clinical signs
- Eye examination
- Laboratory findings

**INTENDED USE**

The FCV ELISA test is designed to detect antibodies against Feline Calici Virus proteins. To this end Feline Calici proteins are attached to the solid phase. The strips are washed after incubation to remove unbound materials. The cat sera to be tested are diluted and added to the wells and incubated. After incubation the strips are washed, a HRPO labeled anti-species conjugate is added to detect bound cat antibodies to FCV. After incubation and rinsing the substrate is added and the optical density is measured at 450 nm.

**PRINCIPLE**

The test is based on the reaction of FCV proteins with polyclonal cat antibodies.

To this end FCV proteins have been attached to the solid phase.

The diluted cat serum/plasma sample is added to the wells of the pre-coated plate.

After washing, the bound cat antibodies are detected by a HRPO conjugated anti-species conjugate.

The color reaction is directly related to the concentration of FCV antibodies in serum/plasma samples.

**CONTENTS**

- 12 x 8 **microtiter strips**.
- 1 x strip holder.
- 1 x 18 mL **ELISA Buffer**.
- 1 x 12 mL **HRPO conjugated anti-species antibodies**.
- 1 x 0.5 mL **Positive Control**.
- 1 x 0.5 mL **Negative Control**.
- 1 x 20 mL Wash-solution (200x concentrated), dilute in de-ionized water before use!
- 1 x 8 mL **Substrate A**.
- 1 x 8 mL **Substrate B**.
- 1 x 8 mL **Stop Solution**.
- 1 x Plastic cover seal.

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The ELISA should be stored at 4-8°C. An unopened package can be used until the expiry date. Avoid repeated freezing and thawing of all the products as this increases non-specific reactivity.

Samples may be used fresh or may be kept frozen below -20°C before use.

Positive and negative controls may be stored in aliquots at -20°C and used until the expiry date.

**WASH PROTOCOL**

In ELISA's, un-complexed components must be removed efficiently between each incubation step. This is accomplished by appropriate washing. It should be stressed that each washing step must be carried out with care to guarantee reproducible inter- and intra-assay results. It is essential to follow the washing procedures outlined below. Washing may be done manually or with automatic equipment. Automatic washing equipment usually gives better results.

**Manual washing**

1. Empty each well by turning the microtiter plate upside down followed by a firm vertical downward movement to remove the buffer.
2. Fill all the wells with 250 µL washing solution.
3. This washing cycle (1 and 2) should be carried out at least 4 times.
4. Turn the plate upside down and empty the wells with a firm vertical downward movement.
5. Place the inverted plate on absorbent paper towels and tap the plate firmly to remove any residual washing solution in the wells.
6. Take care that none of the wells dries out before the next reagent is dispensed.

**Washing with automatic equipment**

When automatic plate washing equipment is used, check that all wells are aspirated completely and that the washing solution is correctly dispensed, reaching the rim of each well during each rinsing cycle. The washer should be programmed to execute at least 4 washing cycles.

**PRECAUTIONS**

- Handle all biological materials as though capable of transmitting FCV.
- Do not pipette by mouth.
- Do not eat, drink, smoke, prepare foods or apply cosmetics within the designated work area.
- TMB is toxic by inhalation, through contact with skin or when swallowed; observe when handling the substrate.
- Do not use components past the expiry date and do not mix components from different serial lots together.
- Optimal results will be obtained by strict adherence to this protocol. Careful pipetting and washing throughout this procedure are necessary to maintain precision and accuracy.
- Each well is ultimately used as an optical cuvette. Therefore, do not touch the under-surface of the microtiter plate and protect it from damage and dirt.

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### TEST PROTOCOL

1. Open the packet of strips and take out the strips to be used. Cover the remaining strips with a part of the provided seal and store them at +4°C and use them within 10 days.  
Wash the microtiter strip(s) with washing solution, according to washing protocol.  
The washing solution provided must be diluted 200x in de-ionized water
2. Divide the positive and negative control into aliquots after opening and store at –20°C until use.
3. Make 3-step dilution of each sample in ELISA buffer, starting 1:30 (90; 270; 810) in a round bottomed microtiter plate.  
Make also a 3-step dilution of the positive and negative control.
4. Transfer 100 µL of this dilution to the FCV coated microtiter strips.  
Seal and incubate for 60 min. at 37°C.
5. Wash as in 1.
6. Dispense 100 µL HRPO conjugated anti-species antibody to all wells.
7. Seal and incubate 60 min. at 37°C.
8. Wash as in 1.
9. Mix equal parts of buffer A and buffer B with gentle shaking. Prepare immediately before use!  
Dispense 100 µL Substrate Solution to each well.  
Incubate 10-20 min. at room temperature (21°C).
10. Add 50 µL Stop Solution to each well; mix well.
11. Read the absorbency values immediately (within 10 min.!) at 450 nm.

### VALIDATION OF THE TEST

In order to confirm appropriate test conditions,  
the weak positive control should give an extinction  $\geq 1.500$  OD and an endpoint titer  $\geq 90$ .  
The negative control should give an OD  $\leq 0.600$  and an endpoint titer  $\leq 30$ .

### INTERPRETATION OF TEST RESULTS

Intermediate antibody titers (90; 270) in diseased animals showing signs suggestive of FCV are considered positive and the cat will be suspected of shedding FCV. A rise in antibody titer in a non-vaccinated cat with a FCV infection represents an exaggerated, effective immune response.

A titer  $\geq 270$  is considered to be protective, under normal virus pressure. If cat density increase viral pressure will also increase and the titer might not be high enough.

In summary:

< 30	=	no antibodies found.
> 90	=	antibodies found, retest in 3 months. (probably shedding FCV)
> 270	=	high titer, considered to be protective.

*The purchaser assumes the entire risk as to the performance of these products.*





















*DRG shall not be liable for indirect, special or consequential damage of any kind resulting from use of these products.*

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### SYMBOLS USED WITH DRG ASSAY'S

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
	European Conformity	CE-Konformitätskennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeitsdatum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità
Symbol	Portugues	Dansk	Svenska	Ελληνικά	
	Consulte as instruções de utilização	Se brugsanvisning	Se bruksanvisningen	Εγχειρίδιο χρήστη	
	Conformidade com as normas europeias	Europeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση	
	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό	
					
	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου	
	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος	
		Indeholder tilstrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις	
	Temperatura de conservação	Opbevarings-temperatur	Förvaringstemperatur	Θερμοκρασία αποθήκευσης	
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης	
	Fabricante	Producent	Tillverkare	Κατασκευαστής	
Distributed by					
Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο	
Volume/No.	Volume/Número	Volumen/antal	Volym/antal	Όγκος/αριθ..	