

1 INTRODUCTION

Canine Para Influenza Virus (PI5) is an important and complex disease (kennel cough) of both wild and domestic dogs. The great majority of dogs that become infected recover completely and develop immunity to PI5. Some of the recovered dogs become carriers of the virus and can infect other dogs. Especially pups can suffer from this complex disease.

The progress of this disease largely depends on other bacteria pasteurella/bordetella or viruses PI3 and Rhino trachites, present at the time of infection. The disease is characterized by coughing, weakness, flowing of tears and sneezing.

Important in the diagnosis of PI5 are:

- clinical history
- clinical signs
- laboratory findings: - antibody detection

This test measures parainfluenza antibodies which are present in the blood or plasma.

Most antibody positive dogs with clinical symptoms (especially those with intermediary titers) are possible virus carriers and may shed PI5.

2 INTENDED USE OF THE TEST KIT

The PI5 ELISA test kit is designed to detect antibodies against PI5 proteins (mostly glycoproteins).

PI5 proteins are attached to the solid phase. After washing the strips are incubated with the dog sera to be tested. The strips are washed after incubation to remove unbound materials. A HRPO labeled anti-species conjugate is added to detect bound dog antibodies to PI5 proteins. After incubation and rinsing the substrate is added and the optical density is measured at 450 nm.

3 PRINCIPLE OF THE TEST KIT

The test is based on the reaction of PI5 proteins (mostly glycoproteins) with polyclonal dog antibodies. To this end PI5 proteins have been coated to a 96-well microtiter plate.

The diluted dog serum/plasma sample is added to the wells of the coated plate. After washing the bound dog antibodies are detected by a HRPO conjugated antispecies conjugate. The color reaction in the wells is directly related to the concentration of PI5 antibodies in the serum/plasma sample.

4 CONTENTS

- 12 x 8-well **microtiter strips**
- 1 x stripholder
- 1 x 12 ml **HRPO-conjugated (IgG) (anti-species) antibody**
- 1 x 1 ml **positive control** (freeze dried)
- 1 x 1 ml **negative control** (freeze dried)
- 1 x 60 ml **wash solution 200 x concentrated**, which must be diluted in deionized water before use!
- 1 x 18 ml **ELISA buffer**
- 1 x 8 ml **substrate buffer A**
- 1 x 8 ml **substrate buffer B**
- 1 x 8 ml **stop solution**
- 1 x plastic cover seal

5 HANDLING AND STORAGE OF SPECIMENS

The ELISA should be stored at 4-8°C. An unopened package can be used until the expiry date.

Avoid repeated freezing and thawing 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 after reconstitution in aliquots at -20°C and used until the expiry date

6 WASHING 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 movement.
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 by a firm vertical movement.
5. Place the inverted plate on absorbent paper towels and tap the plate firmly to remove 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 using automatic plate wash equipment, check that all wells can be 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.

7 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, store 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 200 x in deionized water!
2. Reconstitute directly before use the positive and negative control in 1.0 ml deionized water, divide into aliquots and store immediately at -20°C until use.
3. **Qualitative:** Make a dilution 1:150 of each sample in ELISA buffer in a round bottomed titer plate.
Make a dilution 1:50 of the (weak) positive and negative control.

Quantitative: Make 3-step dilutions 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 these dilutions to the PI5 coated microtiter strips.
Seal and incubate for 60 min. at 37°C.
5. Wash as in 1.
6. Dispense 100 µl conjugated anti-species antibody to all wells.

DRG® Parainfluenza Virus 5 (PI5) IgG (Canine) ELISA (EIA-2491)**Revised 27 Dec. 2005****For Veterinary Use Only**

7. Seal and incubate 60 min. at 37°C.
8. Wash as in 1.
9. Mix equal parts of buffer A and buffer B by gentle shaking. Prepare immediately before use!
Dispense 100 µl substrate solution to each well.
Incubate 15-25 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.

8 VALIDATION OF THE TEST

In order to confirm appropriate test conditions, the weak positive control should give an extinction ≥ 1.000 OD units and an end point titer ≥ 90 .

The negative control should give an OD ≤ 0.5000 and an end point titer ≤ 30 .

To standardize the PI5 ELISA a weak positive control and negative control have to be tested.

The positive control should give an extinction about ≥ 1.000 OD, measured by 450 nm and an end point titer higher than 1:90.

9 INTERPRETATION OF TEST RESULTS

This test can be used in two ways:

a. qualitatively: positive or negative

A sample is scored positive if the OD is higher than 2.5 x OD of the negative control.

b. quantitatively: end point titer

The end point titer of the sample is the dilution which gives an extinction just above 0.300 OD units (450nm)

Antibody titers of 90 and higher in diseased animals showing signs suggestive of PI5 are considered positive and the dog will be suspected of shedding PI5. A rise in antibody titer in a dog with PI5 represents an exaggerated, effective immune response.

In summary:

- | | |
|--------------|--------------------------------------|
| $\leq 30 =$ | no antibodies found |
| 90 - 270 = | antibodies found, retest in 3 months |
| $\geq 810 =$ | high titer of antibodies found. |
| | Diseased animal: suggestive for PI5. |
| | Healthy animal: retest in 3 months. |

10 PRECAUTIONS

- Handle all biological material as though capable of transmitting PI5.
- Do not pipette by mouth.
- Do not eat, drink, smoke or prepare foods, or apply cosmetics within the designated work area.
- TMB is toxic by inhalation, through contact with skin or when swallowed; observe care when handling the substrate.
- Do not use components past the expiry date and do not mix components from different serial lots together.

DRG® Parainfluenza Virus 5 (PI5) IgG (Canine) ELISA (EIA-2491)




















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- 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.

The entire risk as to the performance of these products is assumed by the purchaser. DRG® shall not be liable for indirect, special or consequential damages of any kind resulting from use of the products.

SYMBOLS USED WITH DRG ELISA'S

Symbol	English	Deutsch	Francais	Espanol	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			
	Catalogue number	Katalog-Nr.	Numéro de catalogue	Numero 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	Temperature de conservation	Temperatura de conservacion	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Distributeur	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	Europæisk 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	Opbevaringstemperatur	Förvaringstemperatur	Θερμοκρασία αποθήκευσης	
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης	
	Fabricante	Producent	Tillverkare	Κατασκευαστής	
Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο	
Volume/No.	Volume/Número	Volumen/antal	Volym/antal	Όγκος/αριθ.	