

DRG[®] Canine Distemper Virus IgG (EIA-2478)**Revised 10 Feb. 2009 (Vers. 4.0)****For Veterinary Use Only**

INTRODUCTION

For diagnosis of Canine Distemper Virus (CDV) infection or vaccination control, demonstration of antibody titer is the most commonly used method. Antibodies induced through infection or vaccination are caught by the virus which is attached to the solid phase by use of monoclonal antibodies.

IgG antibody titers above a dilution of 1:150 are considered protected. After reaching peak values within one or two weeks, antibody titers fall back to a threshold level at which they persist. Re-exposure results in an anamnestic response.

Intended USE

The CDV test kit is based on monoclonal antibodies against a common epitope of CDV, which are coated to the solid phase. The distemper virus is attached to the solid phase by the monoclonal antibody. After the attachment of the antigen (distemper virus) sera containing antibodies are able to react with the attached antigen. After the antigen/antibody reaction, the attached can be detected by use of a polyclonal conjugate.

PRINCIPLE

The test is based on the reaction of CDV proteins with CDV antibodies.

To this end purified CDV proteins have been coated to a 96-well microtiter plate.

The diluted dog serum sample is added to the wells of coated plate.

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

The color reaction in the wells is directly related to the concentration of CDV antibodies in the serum.

CONTENTS

- 12 x 8-well **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** serum (freeze-dried)
- 1 x 1 ml **Negative Control** serum (freeze-dried)
- 1 x 20 ml **wash solution, 200 x concentrated. Dilute in deionized 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

Supplies needed (not included)**HANDLING AND STORAGE OF SPECIMENS**

The kit should be stored at 4°C to 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.

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In ELISA's, between each immunological incubation step un-complexed components have to be removed efficiently. 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 advised to carefully follow the washing procedures outlined below. Both manual washing and washing with automatic equipment can be performed. (Automatic washing equipment usually gives better results).

Manual washing

1. Empty each well by turning the microtitre 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 by a firm short 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 washing 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.

TEST PROTOCOL

1. Open the packet of strip(s), take out the strips to be used. Cover the remaining strips with a part of the provided seal; 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. Reconstitute directly before use the positive control in 0.5 ml and the negative control in 1 ml de-ionized water, divide into aliquots, and store immediately at -20 °C until use. Avoid freeze and thaw cycles.
3. Make 3-step dilution of each sample in ELISA Buffer starting 1:30 (1:90; 1:270; 1:810) 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 coated microtiter strips.
Seal and incubate for 60 minutes at 37°C.
5. Wash as in step 1.
6. Dispense 100 µl conjugated anti-species antibody to all wells.
7. Seal and incubate 60 minutes at 37°C.
8. Wash as in step 1.
9. Mix equal parts of buffer A and buffer B with gentle shaking together. Prepare immediately before use!
Dispense 100 µl substrate solution to each well.
Incubate 10-20 min. at room temperature (21°C.). (Make sure that the negative control does not become too dark.)
10. Add 50 µl stop solution to each well; mix well.
11. Read the absorbency values immediately (within 10 min.!) at 450 nm.

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VALIDATION of the Test

The negative control should give an OD < 0.500 (at 450 nm).

The end point titer of the positive control should be between 1:90 and 1:270 according to the instructions for interpretation of test results.

INTERPRETATION

The end point titer of the sample is the dilution that gives an extinction just above 0.350 OD units at 450 nm. Antibody titers of 270 and higher in diseased animals showing signs suggestive of CDV are considered positive.

In summary:

≤ 30	= no antibodies found.
90-270	= antibodies found, retest in 3 months.
≥ 810	= high titer of antibodies found. Diseased animal: suggestive for CDV.
Healthy animal: Retest in 3 months	

PRECAUTIONS

- Handle all biological materials as though capable of transmitting CDV.
- 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, in contact with skin and is swallowed; observe care when handling the substrate.
- Do not use components past expiration 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 prevent 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.

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Symbols used with DRG Assays

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	Europeaisk 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..	