

**DRG® Clostridium piliforme (Rat) EcoELISA (EIA-4954 / EIA-4955)**

**As of 1 May 2009 (Vers. 1.0)**

**For Veterinary Use Only**

**1 LIST OF AVAILABLE TESTS**

Viral/Mycosomal Antibody Detection EcoELISA Kits (Each Parameter with 48 and 96 wells available.)

<b>Parameter</b>	<b>Eco Kit 48 tests REF</b>	<b>EcoDuo Kit 96 tests REF</b>
Adenovirus-FL&K87 (Mouse) EcoELISA	EIA-4946	EIA-4947
Adenovirus-FL&K87 (Rat) EcoELISA	EIA-4948	EIA-4949
Carbacillus (Rat) EcoELISA	EIA-4950	EIA-4951
Clostridium piliforme (Mouse) EcoELISA	EIA-4952	EIA-4953
Clostridium piliforme (Rat) EcoELISA	EIA-4954	EIA-4955
Cytomegalovirus (Mouse) EcoELISA	EIA-4956	EIA-4957
Cytomegalovirus (Guinea Pig) EcoELISA	EIA-4958	EIA-4959
Ectromelia Virus (Mouse) EcoELISA	EIA-4960	EIA-4961
Encephalitozoon cuniculi (Mouse) EcoELISA	EIA-4962	EIA-4963
Encephalitozoon cuniculi (Rat) EcoELISA	EIA-4964	EIA-4965
Encephalitozoon cuniculi (Guinea Pig) EcoELISA	EIA-4966	EIA-4967
Encephalitozoon cuniculi (Hamster) EcoELISA	EIA-4968	EIA-4969
Hantaan Virus (Rat) EcoELISA	EIA-4970	EIA-4971
Hepatitis Virus (Mouse) EcoELISA	EIA-4972	EIA-4973
Corona/Sialodacryoadenitis Virus (Rat) EcoELISA	EIA-4974	EIA-4975
Kilham Virus (Rat) EcoELISA	EIA-4976	EIA-4977
Lymphoc.Choriomeningitis V. (Mouse) EcoELISA	EIA-4978	EIA-4979
Lymphoc.Choriomeningitis V. (Rat) EcoELISA	EIA-4980	EIA-4981
Lymphoc.Choriomeningitis V. (Guinea Pig)	EIA-4982	EIA-4983
Lymphoc. Choriomeningitis V. (Hamster)	EIA-4984	EIA-4985
Minute Virus (Mouse) EcoELISA	EIA-4986	EIA-4987
Mycoplasma pulmonis (Mouse) EcoELISA	EIA-4988	EIA-4989

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<b>Parameter</b>	<b>Eco Kit 48 tests REF</b>	<b>EcoDuo Kit 96 tests REF</b>
Mycoplasma pulmonis (Rat) EcoELISA	EIA-4990	EIA-4991
Mycoplasma pulmonis (Hamster) EcoELISA	EIA-4992	EIA-4993
Parvovirus-1 (Mouse) EcoELISA	EIA-4994	EIA-4995
Parvovirus-1 (Rat) EcoELISA	EIA-4996	EIA-4997
Pneumonia Virus (Mouse) EcoELISA	EIA-4998	EIA-4999
Pneumonia Virus (Rat) EcoELISA	EIA-5000	EIA-5001
Pneumonia Virus (Guinea Pig) EcoELISA	EIA-5002	EIA-5003
Pneumonia Virus (Hamster) EcoELISA	EIA-5004	EIA-5005
Pneumonitis Virus (Mouse) EcoELISA	EIA-5006	EIA-5007
Polio Virus (Mouse) EcoELISA	EIA-5008	EIA-5009
Polio Virus (Rat) EcoELISA	EIA-5010	EIA-5011
Polyoma Virus (Mouse) EcoELISA	EIA-5012	EIA-5013
Reovirus Type 3 (Mouse) EcoELISA	EIA-5014	EIA-5015
Reovirus Type 3 (Rat) EcoELISA	EIA-5016	EIA-5017
Reovirus Type 3 (Guinea Pig) EcoELISA	EIA-5018	EIA-5019
Reovirus Type 3 (Hamster) EcoELISA	EIA-5020	EIA-5021
Rotavirus/EDIM (Mouse) EcoELISA	EIA-5022	EIA-5023
Sendai Virus (Mouse) EcoELISA	EIA-5024	EIA-5025
Sendai Virus (Rat) EcoELISA	EIA-5026	EIA-5027
Sendai Virus (Guinea Pig) EcoELISA	EIA-5028	EIA-5029
Sendai Virus (Hamster) EcoELISA	EIA-5030	EIA-5031
Simian Virus 5 (Guinea Pig) EcoELISA	EIA-5032	EIA-5033
Simian Virus 5 (Hamster) EcoELISA	EIA-5034	EIA-5035
Toolan's H-1 Virus (Rat) EcoELISA	EIA-5036	EIA-5037

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**2 INTRODUCTION**

For the Detection of Viral/Mycosomal Infections in Laboratory Animals.

**For research use only. Not for diagnostic use.**

**3 WARNINGS AND PRECAUTIONS**

1. This kit is for research use only. Not for diagnostic use.
2. Avoid contact with Stop Solution containing 0.5 mol/L H<sub>2</sub>SO<sub>4</sub>. It may cause skin irritation and burns.
3. Never pipette by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
4. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
5. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
6. Handling should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
7. Do not use reagents beyond expiry date as shown on the kit labels.
8. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiter plate readers.
9. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
10. Chemicals and prepared or used reagents have to be treated as hazardous waste according the national biohazard safety guideline or regulation.

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## 4 REAGENTS

### 4.1 Reagents provided

Component		Eco Kit 48 tests	EcoDuo Kit 96 tests
<b>Microtiter Strips</b>	Containing 6 positive and 6 negative antigen coated strips (alternating + [AG] and -[TC] antigen strips)	1 strip holder	2 strip holder
<b>Dilution Buffer</b>	25 mL, ready to use.	1 bottle	2 bottle
<b>HRP Conjugate</b>	11 mL, ready to use	1 vial	2 vial
<b>Positive Control Conjugate</b> (only for Clostridium piliforme (Mouse) EcoELISA EIA-4952 or EIA-4953)	11 mL, ready to use	1 vial	1 vial
<b>Substrate Solution</b>	14 mL, ready to use. Contains Tetramethylbenzidine (TMB).	1 vial	2 vial
<b>Stop Solution</b>	12 mL, ready to use. Contains 0.5M H <sub>2</sub> SO <sub>4</sub> , Avoid contact with the stop solution. It may cause skin irritations and burns.	1 vial	2 vial
<b>Wash Solution 40X</b>	30 mL, concentrate;	1 bottle	1 bottle
<b>Positive Control Serum</b>	0.5 mL	1 vial	1 vial
<b>Negative Control Serum</b>	0.1 ml	1 vial	1 vial

### 4.2 Materials required but not provided

- Calibrated variable precision micropipettes.
- Absorbent paper.
- Distilled or deionized water
- Timer
- A microtiter plate calibrated reader (450 ± 10 nm)

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#### **4.3 Reagent Storage and Preparation**

Bring all reagents and required number of strips to room temperature prior to use.

##### **Microtiter Strips**

The ready-to-use microwell plate is adsorption coated with partially-purified antigen and tissue control (a solution of bovine serum albumin at the same protein concentration as the antigen), resp.

All wells in rows A, C, E, and G are coated with antigen; wells in rows B, D, F, and H are coated with tissue control

Upon receipt store entire microwell plate and remnant removawell-strips at – 20°C or below.

Expiration date is 6 month from date of delivery.

Storage at –70°C will increase shelf life!

##### **Controls**

The sera are supplied diluted in phosphate-buffered saline (PBS) supplemented with 5% w/v bovine serum and 50 µg/ml of gentamycin as a preservative; they have not been heat-inactivated and are non-sterile.

Upon receipt and/or after diluting store at –20°C or below.

Avoid repeated freezing and thawing!

If the volume supplied will not be used at one time, divide it into small aliquots and store at –20°C or below until needed.

Frozen antiserum should retain activity for at least 6 months. Once thawed, store at 4°C for no more than 24 hours.

Dilute the Positive Control antiserum **1:2** with Dilution Buffer.

Dilute the Negative Control antiserum **1:5** with Dilution Buffer.

##### **Wash Solution**

Add deionized water to the 40X concentrated Wash Solution.

Dilute 30 mL of concentrated *Wash Solution* with 1170 mL deionized water to a final volume of 1200 mL.

*The diluted Wash Solution is stable for 2 weeks at room temperature.*

#### **4.4 Disposal of the Kit**

The disposal of the kit must be made according to the national regulations.

#### **4.5 Damaged Test Kits**

In case of any severe damage to the test kit or components, DRG has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

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## 5 SAMPLE PLATE ORGANISATION

A 96-well ELISA plate comprises 8 rows and 12 columns. The rows are designated letter A - H and the columns number 1 – 12.

Individual samples should be identified with a number. Write these numbers in boxes A1 to H11.

Try to start with the lowest number on the top of the column. When a column has been filled, continue in the next column, starting on the top.

Note that each sample is tested in two rows, a test well coated with antigen (labelled AG) and a control well coated with an extract of the tissue, in which the antigen was prepared (labelled TC).

As there are 8 wells in a column, 4 samples can be tested per column. Within these general rules, the organisation may be slightly varied to make the best use of space on the plates.

Reserve column 12 for controls. From the top pair of wells, enter the positive (“high pos”) control, negative (“low pos.”) control and diluent (“neg.”) control.

### 96-well ELISA Plate –Precoated

(Pipetting scheme – Proposal)

			1	2	3	4	5	6	7	8	9	10	11	12
(+)	AG	<b>A</b>	S1	S5	S9	S13	S17	...						CP
(–)	TC	<b>B</b>	S1	S5	S9	S13	S17							CP
(+)	AG	<b>C</b>	S2	S6	S10	...	...							CN
(–)	TC	<b>D</b>	S2	S6	...	...								CN
(+)	AG	<b>E</b>	S3	S7	...									CD
(–)	TC	<b>F</b>	S3	S7										CD
(+)	AG	<b>G</b>	S4	S8										...
(–)	TC	<b>H</b>	S4	S8										...

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### **16-well Strips (“Removawells”) - Precoated**

The 96-well “removawell” plate can be divided into strips containing 2 columns and 8 rows.

As before, one row is coated with antigen (AG) and the other with extract tissue (TC).

Each sample is tested in two adjacent wells of a column. Reserve columns for positive and negative controls.

			<b>1</b>	<b>2</b>
(+)	AG	<b>A</b>	S1	S5
(-)	TC	<b>B</b>	S1	S5
(+)	AG	<b>C</b>	S2	...
(-)	TC	<b>D</b>	S2	...
(+)	AG	<b>E</b>	S3	...
(-)	TC	<b>F</b>	S3	
(+)	AG	<b>G</b>	S4	
(-)	TC	<b>H</b>	S4	

## **6 ASSAY PROCEDURE**

Dilute test sera **1: 60** with Dilution Buffer. 100 µL per diluted sample is required.

Depending on the individual quality of test sera it is recommended to further dilute sample to 1:120 and/or preabsorb unspecific binding moieties by Kaolin pretreatment.

Additionally It is supposed useful to pretreat microwells with 5 % w/v non-fat dry milk in PBS, followed by a wash cycle, prior to adding samples.

### **6.1 Test Procedure**

1. Add **50 µL** of each **diluted sample** to 2 wells of the antigen-coated microwell plate.  
One well (A1, C1, ...) is the antigen test (AG), the other (B1, D1, ...) is the tissue control (TC).
2. Add **50 µL** of the **diluted controls** to the wells. 2 wells per control antiserum (positive and negative; 4 wells).
3. Incubate the filled micro-well-plate at room temperature (RT) for 30 minutes.
4. Fill each well with diluted **wash solution**, tip plate to remove wash solution quickly (rapid rinse). Repeat 3 times.  
Fill each plate with wash solution and remove it after 3 minutes (slow rinse). Repeat 3 times.

Alternatively use an ELISA Washer – 1.5 ml/well (5 x 300 µL/well).

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5. Add **100 µL** of **HRP Conjugate** to all wells. (See NOTE)
  6. Incubate microwell plate at room temperature (RT) for 30 minutes.
  7. Repeat wash procedure, as in step 5, to remove unbound conjugate.
  8. Add **100 µL** of **Substrate Solution** to all wells.
  9. Incubate at room temperature under observation (on a white background) until the negative and/or diluent control wells content starts developing a distinct blueish colour reaction, but not longer than for 20 minutes.
  10. Stop the enzymatic reaction by adding **50 µL** of **Stop Solution** to each well.
  11. Read results visually or determine the absorbance (OD) of each well at **450 ± 10 nm** with a microtiter plate reader.

**NOTE:**

Only for Clostridium piliforme (Mouse) EcoELISA EIA-4952 or EIA-4953

Step 5 is as follows:

5. Add **100 µL** of **HRP Conjugate** to all wells, except positive control wells, **which have to be reacted with supplied Positive Control-Conjugate !!!**

**7 INTERPRETATION**

The absorbance value (AG-TC) is divided by 0.15 to yield a score.

A score	< 1	is <i>negative</i> .
A score from	1 to < 3	is <i>equivocal</i> and the results must be rechecked.
A score of	≥ 3	is <i>positive</i> .

High reading in the TC wells invalidates the test and the respective sample should be repeated by an alternate assay.

**8 LIMITATIONS OF USE**

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice. Any improper handling of samples or modification of this test might influence the results.

**9 LEGAL ASPECTS****9.1 Reliability of Results**

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.



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The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact DRG.

**9.2 Liability**

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.

Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

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### SYMBOLS USED WITH DRG ELISAS

Symbol	English	Deutsch	Français	Español	Italiano
	European Conformity	CE-Konformitätskennzeichnung	Conforme aux normes européennes	Conformidad europea	Conformità europea
	Consult instructions for use	Gebrauchsanweisung beachten	Consultez le Mode d'emploi	Consulte las Instrucciones	Consulti le istruzioni
	In vitro diagnostic device	In-vitro-Diagnostikum	Diagnostic in vitro	Diagnóstico in vitro	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.	Référence	Número de catálogo	No. di Cat.
	Lot. No. / Batch code	Chargen-Nr.	No. de lot	Número de lote	Lotto no
	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	Fabbicante
<i>Distributed by</i>	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
<i>Content</i>	Content	Inhalt	Contenu	Contenido	Contenuto
<i>Volume/No.</i>	Volume / No.	Volumen/Anzahl	Volume/Numéro	Volumen/Número	Volume/Quantità
<i>Microtiterwells</i>	Microtiterwells	Mikrotiterwells	Plaques de micro-titration	Placas multipocillo	Micropozzetti
<i>HRP Conjugate</i>	Enzyme Conjugate	Enzymkonjugat	Conjugué enzymatique	Conjugado enzimático	Tracciante enzimatico
<i>Substrate Solution</i>	Substrate Solution	Substratlösung	Solution substrat	Solución de sustrato	Soluzione di substrato
<i>Stop Solution</i>	Stop Solution	Stopplösung	Solution d'arrêt	Solución de parada	Soluzione d' arresto
<i>Zero Standard</i>	Zero Standard	Nullstandard	Standard 0	Estándar 0	Standard zero
<i>Standard</i>	Standard	Standard	Standard	Estándar	Standard
<i>Control</i>	Control	Kontrolle	Contrôle	Control	Controllo
<i>Pos. Control</i>	Positive Control	Positive Kontrolle	Positif Contrôle	Control positivo	Controllo positivo
<i>Neg. Control</i>	Negative Control	Negative Kontrolle	Négatif Contrôle	Control negativo	Controllo negativo
<i>Cut-off Control</i>	Cut-off Control	Grenzwert-Kontrolle	Valeur limite Contrôle	Control valor limite	Controllo valore limite
<i>Wash Solution</i>	Wash Solution	Waschlösung	Solution de lavage	Solución de lavado	Soluzione di lavaggio
<i>Sample Diluent</i>	Sample Diluent	Probenverdünnungs-medium	Solution pour dilution de l'échantillon	Solución para dilución de la muestra	Diluyente dei campioni
<i>Conjugate Diluent</i>	Conjugate Diluent	Konjugatverdünnungs-medium	Solution pour dilution du conjugué	Solución para dilución del conjugado	Diluyente del tracciante