

**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</b>                | <b>EcoDuo Kit</b>             |
|---|-------------------------------|-------------------------------|
|   | <b>48 tests</b><br><b>REF</b> | <b>96 tests</b><br><b>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                      |

**DRG<sup>®</sup> Toolan's H-1 Virus (Rat) EcoELISA (EIA-5036 / EIA-5037)**

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

**For Veterinary Use Only**

| <b>Parameter</b>                       | <b>Eco Kit<br/>48 tests<br/>REF</b> | <b>EcoDuo Kit<br/>96 tests<br/>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.

**4 REAGENTS**

**4.1 Reagents provided**

| Component   |  | <b>Eco Kit<br/>48 tests</b> | <b>EcoDuo Kit<br/>96 tests</b> |
|---|--|-----------------------------|--------------------------------|
| <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><br>(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.<br>Contains Tetramethylbenzidine (TMB).   | 1 vial                      | 2 vial                         |
| <b>Stop Solution</b>  | 12 mL, ready to use.<br>Contains 0.5M H <sub>2</sub> SO <sub>4</sub> ,<br>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  $-20^{\circ}\text{C}$  or below.

Expiration date is 6 month from date of delivery.

Storage at  $-70^{\circ}\text{C}$  will increase shelf life!

##### Controls

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

Upon receipt and/or after diluting store at  $-20^{\circ}\text{C}$  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  $-20^{\circ}\text{C}$  or below until needed.

Frozen antiserum should retain activity for at least 6 months. Once thawed, store at  $4^{\circ}\text{C}$  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.

|     |    |          | 1  | 2   |
|-----|----|----------|----|-----|
| (+) | 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.





**DRG<sup>®</sup> Toolan's H-1 Virus (Rat) EcoELISA (EIA-5036 / EIA-5037)**

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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                            | Fabbricante                         |
| <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            |