DRG® Respiratory Syncytial Virus (RSV) IgG (EIA-3507)

Revised 27 June 2008 (Vers. 5.0)

Introduction

The **DRG RSV IgG Enzyme Immunoassay Kit** provides materials for the **qualitative** and **semiquantitative** determination of IgG-class antibodies to Respiratory Syncytial Virus (RSV) in serum.
This assay is intended for in vitro diagnostic use only.

PRINCIPLE of the test

The **DRG RSV IgG ELISA** Kit is a solid phase enzyme-linked immunosorbent assay (ELISA)

Microtiter wells as a solid phase are coated with RSV antigen.
Diluted patient specimens and **ready-for-use controls** are pipetted into these wells. During incubation RSV-specific antibodies of positive specimens and controls are bound to the immobilized antigens.
After a washing step to remove unbound sample and control material horseradish peroxidase conjugated anti-human IgG antibodies are dispensed into the wells. During a second incubation this anti-IgG conjugate binds specifically to IgG antibodies resulting in the formation of enzyme-linked immune complexes.
After a second washing step to remove unbound conjugate the immune complexes formed (in case of positive results) are detected by incubation with TMB substrate and development of a blue color. The blue color turns into yellow by stopping the enzymatic indicator reaction with sulfuric acid.
The intensity of this color is directly proportional to the amount of RSV-specific IgG antibody in the patient specimen.
Absorbance at 450 nm is read using an ELISA microtiter plate reader.

Precautions

- This kit is for in vitro diagnostic use only.
- All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
- Controls and Standards has been found to be non-infectious in cell cultures.
- Avoid contact with *Stop Solution* containing 0.5 mol/L H_2SO_4. It may cause skin irritation and burns.
- Never pipette by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
- Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
- Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
- Handling should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
- Do not use reagents beyond expiry date as shown on the kit labels.
- 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.
- 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.
- Chemicals and prepared or used reagents have to be treated as hazardous waste according the national biohazard safety guideline or regulation.

CE

Revised 27 June 2008 (Vers. 5.0)

- For information on hazardous substances included in the kit please refer to Material Safety Data Sheets. Safety Data Sheets for this product are available upon request directly from DRG Instruments GmbH.
  The Safety Data Sheets fit the demands of: EU-Guideline 91/155 EC.

Revised 27 June 2008 (Vers. 5.0)

Kit Components

Contents of the Kit

1. ***Microtiterwells***, 12 x 8 (break apart) strips, 96 wells;
   Wells coated with RSV antigen.
   (incl. 1 strip holder and 1 cover foil)
2. ***Sample Diluent*** ***, 1 vial, 100 mL, ready to use,
   colored yellow; pH 7.2 ± 0.2.
3. ***Pos. Control*** ***, 1 vial, 2.0 mL, ready to use;
   colored yellow, red cap.
4. ***Neg. Control*** ***, 1 vial, 2.0 mL, ready to use;
   colored yellow, yellow cap.
5. ***Cut-off Control*** ***, 1 vial, 2.0 mL, ready to use;
   colored yellow, black cap.
6. ***Enzyme Conjugate*** **, 1 vial, 20 mL, ready to use,
   colored red,
   antibody to human IgG conjugated to horseradish peroxidase.
7. ***Substrate Solution***, 1 vial, 14 mL, ready to use,
   Tetramethylbenzidine (TMB).
8. ***Stop Solution***, 1 vial, 14 mL, ready to use,
   contains 0.5 mol/l H_2SO_4,
   Avoid contact with the stop solution. It may cause skin irritations and burns.
9. ***Wash Solution*** *, 1 vial, 30 mL (20X concentrated for 600 mL), pH 7.2 ± 0.2
   see „Preparation of Reagents“.

* contains 0.03 % ProClin 300
** contains 0.03 % ProClin 300 + 0.01 % Gentamicin sulphate
*** contains 0.03 % ProClin 300 + 0.015 % 5-bromo-5-nitro-1,3-dioxane (BND) +
0.010 % 2-methyl-2H-isothiazol-3-one (MIT)

Equipment and material required but not provided

- A microtiter plate calibrated reader (450/620nm ±10 nm)
  (e.g. the DRG Instruments Microtiter Plate Reader)
- Calibrated variable precision micropipettes
- Incubator 37 °C
- Manual or automatic equipment for rinsing wells
- Vortex tube mixer
- Deionised or (freshly) distilled water
- Timer
- Absorbent paper

Storage and stability of the Kit

Revised 27 June 2008 (Vers. 5.0)

When stored at 2 °C to 8 °C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.
Opened reagents must be stored at 2 °C to 8 °C. Microtiter wells must be stored at 2 °C to 8 °C. Once the foil bag has been opened, care should be taken to close it tightly again.
Opened kits retain activity for four months if stored as described above.

Preparation of Reagents

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

Wash Solution

Dilute *Wash Solution* **1+19** (e.g. 10 mL + 190 mL) with fresh and germ free redistilled water.
Consumption: ~ 5 mL per determination.
Crystals in the solution disappear by warming up to 37 °C in a water bath.
The diluted Wash Solution is stable for 4 weeks at 2 °C to 8 °C.

Disposal of the Kit

The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Material Safety Data Sheets (see chapter 13 of this data sheet).

Damaged Test Kits

In case of any severe damage of the test kit or components, DRG have to be informed written, 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.

SPECIMEN

Serum can be used in this assay.
Do not use haemolytic, icteric or lipaemic specimens.

Specimen Collection

Serum:

Collect blood by venipuncture (e.g. Sarstedt Monovette # 02.1388.001), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time.

Specimen Storage

Specimens should be capped and may be stored for up to 24 hours at 2 °C to 8 °C prior to assaying.
Specimens held for a longer time should be frozen only once at –20 °C prior to assay. Thawed samples should be inverted several times prior to testing.

Specimen Dilution

Prior to assaying dilute each patient specimen **1+100** with *Sample Diluent*;
e.g. 10 µL of specimen + 1 mL of *Sample Diluent* **mix well, let stand for 15 minutes mix well before use.**

Please note: Controls are ready for use and must not be diluted!

Revised 27 June 2008 (Vers. 5.0)

TEST PROCEDURE

General Remarks

- Please read the test protocol carefully before performing the assay. Result reliability depends on strict adherence to the test protocol as described.
- **It is very important to bring all reagents, samples and controls to room temperature before starting the test run!**
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate without splashing accurately to the bottom of wells.
- During incubation cover microtiter strips with foil to avoid evaporation.

Revised 27 June 2008 (Vers. 5.0)

Assay Procedure

Prior to commencing the assay, dilute *Wash Solution*, **prepare patient samples as described in point 5.3**, mix well before pipette and establish carefully the **distribution and identification plan** supplied in the kit for all specimens and controls.

1. Select the required number of microtiter strips or wells and insert them into the holder.

   Please allocate at least:
   1 well (e.g. A1) for the substrate blank,
   1 well (e.g. B1) for the *Neg. Control,*
   2 wells (e.g. C1+D1) for the *Cut-off Control* and
   1 well (e.g. E1) for the *Pos. Control*.

   It is left to the user to determine controls and patient samples in duplicate.

2. Dispense
   100 µL of *Neg. Control* into well B1
   100 µL of *Cut-off Control* into wells C1 and D1
   100 µL of *Pos. Control* into well E1 and
   100 µL of each diluted sample with new disposable tips into appropriate wells.
   Leave well A1 for substrate blank!

3. Cover wells with foil supplied in the kit. Incubate for **60 minutes at 37 °C.**

4. Briskly shake out the contents of the wells.
   Rinse the wells **5 times** with diluted *Wash Solution* (**300 µL per well**). Strike the wells sharply on absorbent paper to remove residual droplets.
   Important note:
   The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!

5. Dispense **100 µL** *Enzyme Conjugate* into each well, **except A1**.

6. Cover wells with foil. Incubate for **30 minutes at room temperature (20 °C to 25 °C).**
   Do not expose to direct sun light!

7. Briskly shake out the contents of the wells.
   Rinse the wells **5 times** with diluted *Wash Solution* (300 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.

8. Add **100 µL** of *Substrate Solution* into all wells.

9. Cover wells with foil. Incubate for **exactly 15 minutes at room temperature (20 °C to 25 °C) in the dark**.

10. Stop the enzymatic reaction by adding **100 µL** of *Stop Solution* to each well.
    Any blue color developed during the incubation turns into yellow.
    Note: Highly positive patient samples can cause dark precipitates of the chromogen!

11. Read the optical density at **450/620 nm** with a microtiter plate reader **within 30 minutes** after adding the *Stop Solution*.

Measurement

Adjust the ELISA microplate or microstrip reader **to zero** using the **substrate blank in well A1**.

If - due to technical reasons - the ELISA reader cannot be adjusted to zero using the substrate blank in well A1, subtract the absorbance value of well A1 from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells **at 450 nm** and record the absorbance values for each control and patient sample in the distribution and identification plan.

Dual wavelength reading using 620 nm as reference wavelength is recommended.

Where applicable **calculate the mean absorbance values** of all duplicates.

Results

Validation of the Test Run

The test run may be considered valid provided the following criteria are met:

Substrate blank in A1:	Absorbance value **lower than 0.100**
***Neg. Control* in B1:**	Absorbance value **lower than 0.200**
***Cut-off Control* in C1/D1 :**	Absorbance value **between 0.350 – 0.550**
***Pos. Control* in E1:**	Absorbance value **greater than 0.600**

Calculation

Mean absorbance value of Cut-off Control [CO]

Calculate the mean absorbance value of the two (2) Cut-off Control determinations (e.g. in C1/D1).

Example: *(0.44 + 0.46) : 2 = 0.45 = CO*

Interpretation

POSITIVE Patient (mean) absorbance values more than 10 % above CO
(Mean $OD_{patient}$ > 1.1 x CO)

GREY ZONE Patient (mean) absorbance values from 10 % above to 10 % below CO
repeat test 2 - 4 weeks later - with new patient samples
(0.9 x CO ≤ Mean $OD_{patient}$ ≤ 1.1 x CO)
Results in the second test again in the grey zone ⇒ **NEGATIVE**

NEGATIVE Patient (mean) absorbance values more than 10 % below CO
(Mean $OD_{patient}$ < 0.9 x CO)

Results in DRG Units [DU]

Revised 27 June 2008 (Vers. 5.0)

$$\frac{\text{Patient (mean) absorbance value x 10}}{\text{CO}} = \text{[DRG Units = DU]}$$

Example: $\frac{1.580 \text{ x } 10}{0.45} = 35 \text{ DU}$

Interpretation of Results

Cut-off value: 10 DU
Grey zone: 9 - 11 DU
Negative: < 9 DU
Positive: > 11 DU

Quality Control

It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.
It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.
If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid.
In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.
After checking the above mentioned items without finding any error contact your distributor or DRG directly.

Assay Characteristics

Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte. It is 100 %.

Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte. It is 100

Limitations of Use

Bacterial contamination or repeated freeze-thaw cycles of the specimen may affect the absorbance values.
In immunocompromised patients and newborns serological data only have restricted value.

Legal Aspects

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.
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.

Therapeutical Consequences

Therapeutical consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under point 11.1. Any laboratory result is only a part of the total clinical picture of a patient. Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data.
Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutical consequences be derived.
The test result itself should never be the sole determinant for deriving any therapeutical consequences.

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.
Claims submitted due to customer misinterpretation of laboratory results subject to point 11.2. are also invalid. 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.

REFERENCES

1. Wiegand,R.:Respiratory Syncytial Virus (RSV).In:Brandis,H.,W.Köhler,H.J.Eggers,G.Pulverer (ed.):Med. Mikrobiologie 1994.Gustav Fischer Verlag Stuttgart,Jena,New York (1994) 767-769
2. Hall, C.B., et Immunity to and frequency of reinfection with Respiratory Syncytial Virus. J. Infect. Dis. 163 (1991) 693-698
3. Hohlberg, C.J., et al.: Risk factors for Respiratory Syncytial Virus associated lower respiratory illness in the year of life. Am. J. Epidemiol. 133 (1991) 1135-1151
4. Meurmann, O., et al.: Diagnosis of Respiratory Syncytial Virus infection in children: comparison of viral antigen detection and serology. J. Med. Virol. 14 (1984) 61-65
5. Schumacher, R. F.: Die RSV-Infektion des Frühgeborenen: Nosologie und nosokomiale Epidemiologien. Diss., Freiburg (1990)

Revised 27 June 2008 (Vers. 5.0)

Symbols used with DRG ELISAs

Symbol	English	Deutsch	Francais	Espanol	Italiano
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	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
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Diagnostic in vitro	Diagnóstico in vitro	Diagnostica in vitro
REF	Catalogue number	Katalog-Nr.	Référence	Número de catálogo	No. di Cat.
LOT	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
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Contenu	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Numéro	Volumen/Número	Volume/Quantità
Microtiterwells	Microtiterwells	Mikrotiterwells	Plaques de micro-titration	Placas multipocillo	Micropozzetti
Enzyme Conjugate	Enzyme Conjugate	Enzymkonjugat	Conjugué enzymatique	Conjugado enzimático	Tracciante enzimatico
Substrate Solution	Substrate Solution	Substratlösung	Solution substrat	Solución de sustrato	Soluzione di substrato
Stop Solution	Stop Solution	Stopplösung	Solution d'arrêt	Solución de parada	Soluzione d´arresto
Zero Standard	Zero Standard	Nullstandard	Standard 0	Estándar 0	Standard zero
Standard	Standard	Standard	Standard	Estándar	Standard
Control	Control	Kontrolle	Contrôle	Control	Controllo
Pos. Control	Positive Control	Positive Kontrolle	Positif Contrôle	Control positivo	Controllo positivo
Neg. Control	Negative Control	Negative Kontrolle	Négatif Contrôle	Control negativo	Controllo negativo
Cut-off Control	Cut-off Control	Grenzwert-Kontrolle	Valeur limite Contrôle	Control valor limite	Controllo valore limite
Wash Solution	Wash Solution	Waschlösung	Solution de lavage	Solución de lavado	Soluzione di lavaggio
Sample Diluent	Sample Diluent	Probenverdünnungs-medium	Solution pour dilution de l'échantillon	Solución para dilución de la muestra	Diluente dei campioni
Conjugate Diluent	Conjugate Diluent	Konjugatverdünnungs-medium	Solution pour dilution du conjugué	Solución para dilución del conjugado	Diluente del tracciante

CE

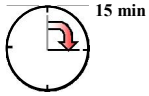
Revised 27 June 2008 (Vers. 5.0)

Short Instructions for Use

	All reagents and specimens must be allowed to come to room temperature (18-25°C) before use.
	Leave well A1 for substrate Blank. Dispense 100 µl of Controls into appropriate wells.
	Dispense 100 µl of sample into selected wells. **(Please note special sample treatment, point 5.3!)**
 60 min	Cover wells with foil. Incubate for **60 minutes at 37 °C**.
	Briskly shake out the contents of the wells.
	Rinse the wells **5 times** with diluted Wash Solution (300 µl per well).
	Strike the wells sharply on absorbent paper to remove residual droplets.
	Dispense 100 µl of Enzyme-Conjugate into each well.
 30 min	Incubate for **30 minutes** at room temperature.
	Briskly shake out the contents of the wells.
	Rinse the wells **5 times** with diluted Wash Solution (300 µl per well).
	Strike the wells sharply on absorbent paper to remove residual droplets.
	Add 100 µl of Substrate Solution to each well.

CE

Revised 27 June 2008 (Vers. 5.0)

	Incubate for **15 minutes** at room temperature.
	Stop the reaction by adding 100 µl of Stop Solution to each well.
	Determine the absorbance of each well at 450 nm.