

Revised 16 Sept. 2010 rm (Vers. 4.1)

USA: RUO

Please use only the valid version of the package insert provided with the kit.

1 INTENDED USE

Third generation Enzyme Immunoassay (ELISA) for determination of antibodies to Hepatitis E Virus in human plasma and sera.

For Research Use Only.

2 PRINCIPLE OF THE TEST

Microplates are coated with HEV-specific synthetic antigens encoding for conservative and immunodominant determinants derived from Mexican and Burma virus strains.

The solid phase is first treated with the diluted sample and HEV Ab are captured, if present, by the antigens.

After washing out all the other components of the sample, in the 2nd incubation bound HEV antibodies, IgG and IgM as well, are detected by the addition of polyclonal specific anti hIgG&M antibodies, labelled with peroxidase (HRP).

The enzyme captured on the solid phase, acting on the substrate/chromogen mixture, generates an optical signal that is proportional to the amount of anti HEV antibodies present in the sample.

A cut-off value let optical densities be interpreted into HEV antibody negative and positive results.

3 COMPONENTS

The kit contains reagents for 96 tests.

1. **Microplate** MICROPLATE 1 microplate,
12 strips of 8 microwells coated with HEV specific synthetic antigens derived from ORF2 and ORF3 regions. Plates are sealed into a bag with desiccant.
2. **Negative Control** CONTROL - 1 x 2.0 ml/vial
Ready to use control. It contains 1% goat serum proteins, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.5% Tween 20, 0.09% Na-azide and 0.1% Kathon GC as preservatives.
The negative control is olive green colour coded.
3. **Positive Control** CONTROL + 1 x 2.0 ml/vial
Ready to use control. It contains 1% goat serum proteins, human antibodies positive to HEV, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.5% Tween 20, 0.09% Na-azide and 0.1% Kathon GC as preservatives. The Positive Control is dark green colour coded.
4. **Calibrator** CAL 1 vial
Lyophilized calibrator. To be dissolved with the volume of EIA grade water reported on the label. It contains foetal bovine serum proteins, human antibodies to HEV whose content is calibrated on 1st WHO reference reagent for HEV antibody, NIBSC code 95/584 at 4+/-10% IU/ml, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.3 mg/ml gentamicine sulphate and 0.1% Kathon GC as preservatives.
Note: The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label.

Revised 16 Sept. 2010 rm (Vers. 4.1)

USA: **RUO**

5. **Wash buffer concentrate 20X** **WASHBUF 20x** 1 x 60 ml/bottle.
20x concentrated solution.
Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.1% Kathon GC.
6. **Enzyme Conjugate** **CONJ** 1 x 16 ml/vial.
Ready to use and red colour coded reagent. It contains Horseradish Peroxidase conjugated goat polyclonal antibodies to human IgG and IgM, 5% BSA, 10 mM Tris buffer pH 6.8+/-0.1, 0.1% Kathon GC and 0.02% gentamicine sulphate as preservatives.
7. **Chromogen/Substrate TMB** **SUBS TMB** 1 x 16 ml/vial.
Ready-to-use component. It contains 50 mM citrate-phosphate buffer pH 3.5-3.8, 4% dimethylsulphoxide, 0.03% tetra-methyl-benzidine or TMB and 0.02% hydrogen peroxide or H₂O₂.
Note: To be stored protected from light as sensitive to strong illumination.
8. **Assay Diluent** **DILAS** 1 x 8 ml/vial.
10 mM tris buffered solution pH 8.0 +/-0.1 containing 0.1% Kathon GC for the pre-treatment of samples and controls in the plate, blocking interference.
9. **Stop Solution** **H₂SO₄ 0.3M** 1 x 15 ml/vial
Contains 0.3M H₂SO₄ solution.
Attention: Irritant (Xi R36/38; S2/26/30)
10. **Sample Diluent** **DILSPE** 1 x 50 ml/vial.
It contains 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.5% Tween 20, 0.09% Na-azide and 0.1% Kathon GC as preservatives. To be used to dilute the sample.
Note: The diluent changes colour from olive green to dark bluish green in the presence of sample.
11. **Plate sealing foils** n° 2
12. **Package insert** n° 1

4 MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (200µl and 10µl) and disposable plastic tips.
2. EIA grade water (bidistilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator capable to provide a temperature of +37°C.
6. Calibrated ELISA microwell reader with 450nm (reading) and possibly with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

5 SPECIMEN: PREPARATION AND RECOMMENDATIONS

1. Blood is drawn aseptically by venipuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis.
No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
2. Avoid any addition of preservatives to samples; especially sodium azide as this chemical would affect the enzymatic activity of the conjugate, generating false negative results.

Revised 16 Sept. 2010 rm (Vers. 4.1)**USA: RUO**

3. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. When the kit is used for the screening of blood units, bar code labeling and electronic reading is strongly recommended.
4. Haemolysed (red) and visibly hyperlipemic (“milky”) samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
5. Sera and plasma can be stored at +2-8°C for up to five days after collection.
For longer storage periods, samples can be stored frozen at –20°C for several months.
Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.
6. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8 µ filters to clean up the sample for testing.

6 PREPARATION OF COMPONENTS AND WARNINGS

A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-use of the device and up to 6 months.

1. Microplates:

Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant is not turned to dark green, indicating a defect of conservation. In this case call DRG’s customer service.

Unused strips have to be placed back into the aluminium pouch, in presence of desiccant supplied, firmly zipped and stored at +2-8°C.

When opened the first time, residual strips are stable till the indicator of humidity inside the desiccant bag turns from yellow to green.

2. Negative Control: Ready to use. Mix well on vortex before use.

3. Positive Control:

Ready to use. Mix well on vortex before use. Handle this component as potentially infective, even if HEV, eventually present in the control, has been chemically inactivated.

4. Calibrator:

Dissolve carefully the content of the lyophilized vial with the volume of EIA grade water reported on its label. Mix well on vortex before use.

Handle this component as potentially infective, even if HEV, eventually present in the control, has been chemically inactivated.

Note: When dissolved the Calibrator is not stable. Store in aliquots at –20°C.

Revised 16 Sept. 2010 rm (Vers. 4.1)**USA: RUO****5. Wash buffer concentrate:**

The whole content of the 20x concentrated solution has to be diluted with bidistilled water up to 1200 ml and mixed gently end-over-end before use.

Once diluted, the wash solution is stable for 1 week at +2-8°C. In the preparation avoid foaming as the presence of bubbles could give origin to a bad washing efficiency.

Note: Once diluted, the wash solution is stable for 1 week at +2-8°C.

6. Enzyme conjugate:

Ready to use. Mix well on vortex before use.

Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.

If this component has to be transferred use only plastic, possibly sterile disposable containers.

7. Chromogen/Substrate:

Ready to use. Mix well on vortex before use.

Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.

Do not expose to strong illumination, oxidizing agents and metallic surfaces.

If this component has to be transferred use only plastic, possible sterile disposable container.

8. Assay Diluent:

Ready to use. Mix well on vortex before use.

9. Stop solution:

Ready to use. Mix well on vortex before use.

Attention: Irritant (Xi R36/38; S2/26/30)

Legenda: R 36/38 = Irritating to eyes and skin. S 2/26/30 = In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

10. Sample Diluent:

Ready to use. Mix well on vortex before use.

7 INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (household alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained in order to show a precision of 1% and a trueness of +/-2%. Decontamination of spills or residues of kit components should also be carried out regularly.
2. The ELISA incubator has to be set at +37°C (tolerance of +/-0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The ELISA washer is extremely important to the overall performances of the assay. The washer must be carefully validated and correctly optimized using the kit controls and reference panels, before using the kit for routine laboratory tests. Usually 4-5 washing cycles (aspiration + dispensation of 300 µl/well of washing solution = 1 cycle) are sufficient to ensure that the assay performs as expected. A soaking time of 20 - 30 seconds between cycles is suggested. In order to set correctly their number, it is recommended to run an assay with the kit controls and well characterized negative and positive reference samples, and check to match the values reported below in the section "Internal Quality Control". Regular calibration of the volumes delivered by, and maintenance (decontamination and cleaning of needles) of the washer has to be carried out according to the instructions of the manufacturer.
4. Incubation times have a tolerance of ± 5%.

Revised 16 Sept. 2010 rm (Vers. 4.1)**USA: RUO**

5. The ELISA microplate reader has to be equipped with a reading filter of 450nm and ideally with a second filter (620-630nm) for blanking purposes. Its standard performances should be
 - (a) bandwidth ≤ 10 nm;
 - (b) absorbance range from 0 to ≥ 2.0 ;
 - (c) linearity to ≥ 2.0 ; repeatability $\geq 1\%$.Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer's instructions.
6. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the section "Internal Quality Control". The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of ELISA automated work station is recommended for blood screening when the number of samples to be tested exceed 20 - 30 units per run.

8 PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label of the kit box. Do not use if expired.
2. Check that the liquid components are not contaminated by naked-eye visible particles or aggregates. Check that the Chromogen/Substrate is colorless or pale blue by aspirating a small volume of it with a sterile transparent plastic pipette. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box. Check that the aluminum pouch, containing the microplate, is not punctured or damaged.
3. Dilute all the content of the 20x concentrated Wash Solution as described above.
4. Dissolve the Calibrator as described above.
5. Allow all the other components to reach room temperature (about 1 hr) and then mix as described.
6. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as found in the validation of the instrument for its use with the kit.
7. Check that the ELISA reader has been turned on at least 20 minutes before reading.
8. If using an automated workstation, turn it on, check settings and be sure to use the right assay protocol.
9. Check that the micropipettes are set to the required volume.
10. Check that all the other equipment is available and ready to use.
11. In case of problems, do not proceed further with the test and advise the supervisor.

9 ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.

Revised 16 Sept. 2010 rm (Vers. 4.1)

USA: **RUO**

9.1 Automated assay:

In case the test is carried out automatically with an ELISA system, we suggest to make the instrument aspirate 200 µl Sample Diluent and then 10 µl sample.

All the mixture is then carefully dispensed directly into the appropriate sample well of the microplate. Before the next sample is aspirated, needles have to be duly washed to avoid any cross-contamination among samples.

Do not dilute controls/calibrator as they are ready to use.

Dispense 200 µl controls/calibrator in the appropriate control/calibration wells.

Important Note: Visually monitor that samples have been diluted and dispensed into appropriate wells. This is simply achieved by checking that the color of dispensed samples has turned to dark bluish-green while the color of the negative control has remained olive green.

For the next operations follow the operative instructions reported below for the Manual Assay.

It is strongly recommended to check that the time lap between the dispensation of the first and the last sample will be calculated by the instrument and taken into consideration by delaying the first washing operation accordingly.

9.2 Manual assay:

1. Place the required number of Microwells in the microwell holder. Leave the 1st well empty for the operation of blanking.
2. Dispense 200 µl of Negative Control in triplicate, 200 µl Calibrator in duplicate and 200 µl Positive Control in single in proper wells.
Do not dilute Controls and Calibrator as they are pre-diluted, ready to use !
3. Add 200 µl of Sample Diluent to all the sample wells;
then dispense 10 µl sample in each properly identified well.
Mix gently the plate, avoiding overflowing and contaminating adjacent wells, in order to fully disperse the sample into its diluent.

Important note: Check that the color of the Sample Diluent, upon addition of the sample, changes from light green to dark bluish green, monitoring that the sample has been really added.

4. Dispense 50 µl Assay Diluent into all the controls/calibrator and sample wells.
Check that the color of samples has turned to dark blue.
5. Incubate the microplate for **45 min at +37°C**.

Important note: Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using ELISA automatic instruments.

6. Wash the microplate with an automatic washer by delivering and aspirating 300 µl/well of diluted washing solution as reported previously.
7. Pipette 100 µl Enzyme Conjugate into each well, except the 1st blanking well, and cover with the sealer. Check that this red colored component has been dispensed in all the wells, except A1.

Revised 16 Sept. 2010 rm (Vers. 4.1)**USA: RUO**

Important note: Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur.

8. Incubate the microplate for **45 min at +37°C**.
9. Wash microwells as in step 6.
10. Pipette 100 µl Chromogen/Substrate mixture into each well, the blank well included.
Then incubate the microplate **at room temperature (18-24°C) for 15 minutes**.

Important note: Do not expose to strong direct illumination. High background might be generated.

11. Pipette 100 µl Stop Solution (Sulphuric Acid) into all the wells using the same pipetting sequence as in step 10 to stop the enzymatic reaction.
Addition of acid will turn the positive control and positive samples from blue to yellow.
12. Measure the color intensity of the solution in each well, as described in section 9.5, at 450 nm filter (reading) and possibly at 620-630 nm (background subtraction), blanking the instrument on A1.

Important notes:

1. If the second filter is not available ensure that no finger prints are present on the bottom of the microwell before reading at 450nm. Finger prints could generate false positive results on reading.
2. Reading has to be carried out just after the addition of the Stop Solution and anyway not any longer than 20 minutes after its addition. Some self oxidation of the chromogen can occur leading to high background.

Revised 16 Sept. 2010 rm (Vers. 4.1)

USA: **RUO**

10 ASSAY SCHEME

Method	Operations
Controls & Calibrator	200 µl
Samples	200 µl dil.+10 µl
Assay Diluent	50 µl
1st incubation	45 min
Temperature	+37°C
Wash step	4-5 cycles
Enzyme conjugate	100 µl
2nd incubation	45 min
Temperature	+37°C
Wash step	4-5 cycles
TMB/H ₂ O ₂	100 µl
3rd incubation	15 min
Temperature	r.t.
Stop Solution	100 µl
Reading OD	450 nm

An example of dispensation scheme is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S2										
B	NC	S3										
C	NC	S4										
D	NC	S5										
E	CAL	S6										
F	CAL	S7										
G	PC	S8										
H	S1	S9										

Legenda: BLK = Blank NC = Negative Control
CAL = Calibrator PC = Positive Control S = Sample



DRG[®] HEV Ab ELISA (EIA-4416)



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11 CALCULATION OF THE CUT-OFF

The test results are calculated by means of the mean OD450nm value of the Negative Control (NC) and a mathematical calculation, in order to define the following cut-off formulation:

$$\text{Cut-Off} = \text{NC mean OD450nm} + 0.350$$











The value found for the test is used for the interpretation of results as described in the next paragraph.

Important note: When the calculation of results is done by the operative system of an ELISA automated work station be sure that the proper formulation is used to calculate the cut-off value and generate the right interpretations of results.

12 REFERENCES / LITERATURE

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Revised 16 Sept. 2010 rm (Vers. 4.1)
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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	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	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità