DRG® HTLV I&II Ab (EIA-3905)

Revised 23 July 2012 rm (Vers. 4.1) **RUO in the USA**

This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

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

INTENDED USE

Enzyme Immuno Assay (ELISA) for measurement of antibodies to Human T-cell Lymphotropic Virus type I&II or HTLV I&II Ab.

PRINCIPLE OF THE TEST

Microplates are coated with HTLV I&II specific synthetic immunodominant antigens derived from gp46-I, gp46-II and gp21-I.
The solid phase is first treated with the sample and anti HTLV I&II Ab are captured, if present, by the antigens coated on the microplate.
After washing out all the other components of the sample, in the second incubation bound anti HTLV I&II total antibodies, are detected by the additionof specific synthetic antigens derived from gp46-I, gp46II and gp21, 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 HTLV I&II antibodies present in the sample. After blocking the enzymatic reaction, its optical density is measured by an ELISA reader.
The version is particularly suitable for automated screenings.

COMPONENTS

The kit contains sufficient reagents to perform 192 tests.

1. Microplate MICROPLATE
n° 2 microplates. 12 strips of 8 breakable wells.
Microplates are coated with HTLV I&II specific synthetic immunodominant antigens derived from gp46-I, gp46-II and gp21-I. Plates are sealed into a bag with desiccant. Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 4 °C.

2. Low Control CONTROL –
1 x 4.0 mL/vial. Ready to use control.
It contains 5% BSA, 10mM phosphate buffer pH 7.4+/-0.1, 0.09% sodium azide and 0.1% Kathon GC.
Brown/yellow colour coded

3. High Control CONTROL +
1 x 4.0 mL/vial. Ready to use control.
It contains 5% BSA, 10mM phosphate buffer pH 7.4+/-0.1, 0.09% sodium azide, inactivated human serum high to HTLV

Ab, 0.1% Kathon GC.
Green colour coded

4. Calibrator CAL …ml.
n° 2 vials. Lyophilized calibrator.
It contains inactivated anti HTLV I&II antibodies, calibrated agains Seracare Accurun 24, 4% Bovine serum albumin, 2% Mannitol, 50mM Tris buffer pH 7.8, 0.2 mg/ml gentamicine sulphate and 0.1% Kathon GC.
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.

5. Wash buffer concentrate WASHBUF 20X
2 x 60 mL/bottle. 20x concentrated solution
containing 0.1% Kathon GC as preservative.
Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2 and 0.05% Tween 20 .

6. Enzyme Conjugate CONJ
1 x 25 mL/cassette. Ready-to-use solution.
It contains HTLV synthetic antigens mixture, labelled with HRP, 5% BSA, 10 mM Tris buffer pH 6.8+/-0.1, 0.3 mg/mL gentamicine sulphate and 0.1% Kathon GC as preservatives. Red color coded

7. Chromogen/Substrate SUBS TMB
1 x 25 mL/cassette. 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 H_2O_2.
Note: To be stored protected from light as sensitive to strong illumination.

8. Sulphuric Acid H_2SO_4 0.3 M
1 x 25 mL/vial. It contains 0.3 M H_2SO_4 solution.
Attention: Irritant (Xi R36/38; S2/26/30).

9. Plate sealing foils n° 4

10. Caps n° 3
Plastic cap to be used to firmly seal the container after first use.

11. Package insert n° 1

MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (200 µl 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 450 nm (reading) and possibly with 620-630 nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

WARNINGS AND PRECAUTIONS

1. The kit has to be used by skilled and properly trained technical personnel only. .
2. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
3. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
4. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen/Substrate from strong light and avoid vibration of the bench surface where the test is undertaken.
5. Upon receipt, store the kit at 2 - 8°C into a temperature controlled refrigerator or cold room.
6. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
7. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit replacement.
8. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample.
9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one.
10. Do not use the kit after the expiration date stated on the external container and internal (vials) labels.
11. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
12. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid cross contamination.
13. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated before waste. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..
14. Accidental spills from samples and operations have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.

15. The Sulphuric Acid is an irritant. In case of spills, wash the surface with plenty of water. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

SPECIMEN: PREPARATION AND WARNINGS

1. Blood is drawn aseptically by venipuncture and plasma or serum is prepared using standard techniques of preparation of samples for research 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 low results.
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 °C – 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.

PREPARATION OF COMPONENTS AND WARNINGS

Microplates:
Allow the microplate to reach room temperature (about 1 hour) before opening the container. Check that the desiccant has not turned green, indicating a defect in conservation. In this case call the customer service. Unused strips have to be placed back into the aluminum pouch, with the desiccant supplied, firmly zipped and stored at 2 °C - 8 °C. After first opening, remaining strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.

Low and High Control:
Ready to use. Mix well on vortex before use.

Calibrator:
Add the volume of ELISA grade water, reported on the label, to the lyophilised powder; let fully dissolve and then gently mix on vortex.
Note: *The calibrator after dissolution is not stable. Store frozen in aliquots at –20 °C.*

Wash buffer concentrate:
The whole content of the concentrated solution has to be diluted 20x with bidistilled water and mixed gently end-over-end before use.
During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.
Note: *Once diluted, the wash solution is stable for 1 week at 2 °C - 8 °C.*

Enzyme conjugate:
Ready to use. Mix well on vortex before use.
Avoid contamination of the liquid with oxidising chemicals, dust or microbes. If this component has to be transferred, use only plastic, and if possible, sterile disposable containers..

Chromogen/Substrate:
Ready to use. Mix well on vortex before use.
Avoid contamination of the liquid with oxidising chemicals, airdriven dust or microbes. Do not expose to strong light, oxidising agents and metallic surfaces.
If this component has to be transferred use only plastic, and if possible, sterile disposable container.

Sulphuric Acid:
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.

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 350 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 low and high reference samples, and check to match the values reported below in the sections "Validation of Test" and "Assay Performances". 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%.
5. The ELISA microplate reader has to be equipped with a reading filter of 450 nm and ideally with a second filter (620-630 nm) 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 ≥ 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 sections "Validation of Test" and "Assay Performances". 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 stations is recommended for blood screening when the number of samples to be tested exceed 20-30 units per run.
7. When using automatic devices, in case the vial holder of the instrument does not fit with the vials supplied in the kit, transfer the solution into appropriate containers and label them with the same label peeled out from the original vial. This operation is important in order to avoid mismatching contents of vials, when transferring them. When the test is over, return the secondary labeled containers to 2 °C – 8 °C, firmly capped.

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.

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.

Automated assay

In case the test is carried out automatically with an ELISA system, we suggest to dispense the sample 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 100 µL controls/calibrator in the appropriate control/calibration wells.
Important Note: *Visually monitor that samples have been dispensed into appropriate wells.*
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.
In case an automatic workstation is used, first assure that the instrument is validated according to section 9.6.

Manual assay

1. Place the required number of microwells in the microwell holder.
   Store the other strips into the bag in presence of the desiccant at 2 °C - 8 °C, sealed.
   Leave A1 well empty for the operation of blanking.
2. Dispense 100 µL of Low Control in triplicate,
   100 µL of Calibrator in duplicate and
   100 µL High Control in single in proper wells.
   followed by 100 uL of each of sample.
   Do not dilute Controls and Calibrator as they are pre-diluted, ready to use !

   Check for the presence of samples in wells by naked eye (there is a marked color difference between empty and full wells) or by reading at 450/620 nm. (samples show OD values higher than 0.100).
   .
   Important note:
   Strips have to be sealed with the adhesive sealing foil, only when the test is performed manually.
   Do not cover strips when using ELISA automatic instruments.

3. Incubate the microplate for **45 min at 37 °C**.
4. Wash the microplate with an automatic washer as reported in section 9.3).
5. Pipette 100 µL Enzyme Conjugate into each well, except the 1st blanking well, and cover with the sealer. Check that this red coloured component has been dispensed in all the wells, except A1.

   Important note:
   Be careful not to touch the inner surface of the well with the pipette tip when the conjugate is dispensed.
   Contamination might occur.

6. Incubate the microplate for **45 min at +37 °C**.
7. Wash the microplate with an automatic washer as in step 4.
8. Pipette 100 µL TMB/H_2O_2 mixture into each well, the blank well included.
   Check that the reagent has been correctly added.

   Then incubate the microplate at **room temperature (18 °C - 24 °C) for 15 minutes**.

   Important note:
   Do not expose to strong direct light as a high background might be generated.

9. Pipette 100 µL Sulphuric Acid into all the wells using the same pipetting sequence as in step 8 to stop the enzymatic reaction.
   Addition of acid will turn the high control and high samples from blue to yellow.
10. Measure the color intensity of the solution in each well, as described in section 9.5, with a microplate reader at 450 nm (reading) and possibly at 620-630 nm (background subtraction), blanking the instrument on A1 well.

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 450 nm. Finger prints could generate false high 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.*
3. *Shaking at 350 ± 150 rpm during incubation has been proved to increase the sensitivity of the assay of about 20%.*

ASSAY SCHEME

Method	Operations
Controls & Calibrator Samples	100 µL 100 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_2O_2	100 µL
3rd incubation	**15 min**
Temperature	r.t.
Sulphuric Acid	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 = Low Control CAL = Calibrator POS = High Control S = Sample

INTERNAL QUALITY CONTROL

A check is carried out on the controls and the calibrator any time the kit is used in order to verify whether their OD450 nm values are as expected and reported in the table below.

Check	Requirements
Blank well	< 0.100 OD 450 nm value
Low Control (NC)	< 0.150 mean OD 450 nm value after blanking
Calibrator (CAL)	S/Co ≥ 1.5
High Control (PC)	> 1.000 OD 450 nm value

If the results of the test match the requirements stated above, proceed to the next section.
If they do not, do not proceed any further and operate as follows:

Problem	Check
Blank well > 0.100 OD 450 nm	1. that the Chromogen/Sustrate solution has not got contaminated during the assay
Low Control (NC) > 0.150 OD 450 nm after blanking	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (dispensation of high control instead of low control); 4. that no contamination of the low control or of their wells has occurred due to high samples, to spills or to the enzyme conjugate; 5. that micropipettes haven't got contaminated with high samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.
Calibrator S/Co < 1.5	1. that the procedure has been correctly executed; 2. that no mistake has been done in its distribution (ex.: dispensation of low control instead of calibrator); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.

High Control < 1.000 OD 450 nm	1. that the procedure has been correctly executed; 2. that no mistake has been done in the distribution of controls (dispensation of low control instead of high control. In this case, the low control will have an OD 450 nm value > 0.150, too. 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the high control has occurred.

Should these problems happen, after checking, report any residual problem to the supervisor for further actions.

CALCULATION OF THE Calibrator

The tests results are calculated by means of a cut-off value determined with the following formula on the mean OD450nm value of the Low Control (NC):

NC + 0.200 = Calibrator (Calb)

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.*

LIMIT OF DETECTION

The limit of detection of the assay has been calculated by means of the preparation Accurun 24, lot # 118956, produced by Seracare Life Sciences, USA.
The table below reports the results obtained for this material with three lots of products (P1, P2 and P3) Accurun 24 was diluted in HTLV Ab low serum and examined in 4 replicates.

ACCURUN 24

Dilution	P1	P2	P3
	OD 450nm	OD 450nm	OD 450nm
4x	2.981	2.957	3.455
8x	1.964	1.856	1.992
16x	0.935	0.820	0.971
32x	0.551	0.453	0.562
64x	0.318	0.357	0.434
128x	0.201	0.195	0.251
diluent	0.040	0.059	0.055

The product shows an analytical sensitivity better than the reference kit of previous generation.

REFERENCES

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