DRG

DRG® L1-CAM Elisa (EIA-5074)

Revised 17 Mar. 2010 RM (Vers. 2.0)

RUO

1 INTRODUCTION

1.1 Intended Use

The **DRG L1-CAM ELISA** is an enzyme immunoassay for the quantitative *in vitro* measurement of L1-CAM in human serum.

1.2 Summary and Explanation

L1-is a cell adhesion molecule (CD171) that has thus far proved to be a valuable and powerful marker for the diagnosis and prognosis of ovarian and endometrial tumors. As was shown in many human trials, L1 is a sensitive and specifc marker, pointing out the cell´s origin. L1 provides diagnostic and prognostic information that guides the clinician towards the optimal treatment of the disease. L1 traits clearly suggest that it may also be an efficient therapeutic agent for ovarian and endometrial tumors.

2 PRINCIPLE OF THE TEST

The DRG L1-CAM ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle.

The microtiter wells are coated with a monoclonal/ [mouse] antibody directed towards a unique antigenic site on a L1-CAM molecule. An aliquot of patient sample containing endogenous L1-CAM is incubated in the coated well with enzyme conjugate, which is a biotinylated monoclonal anti- 1-CAM antibody. After incubation the unbound conjugate is washed off. A second incubation with enzyme complex (streptavidin peroxidase) and additional washing follows.

The amount of bound biotin-streptavidin complex is proportional to the concentration of L1-CAM in the sample.

Having added the substrate solution, the intensity of colour developed is proportional to the concentration of L1-CAM in the patient sample.

3 WARNINGS AND PRECAUTIONS

1. This kit is for in vitro use only. For professional use only.
2. 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.
3. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
4. The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch and used in the frame provided.
5. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
6. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
7. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
8. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
9. Allow the reagents to reach room temperature (21-26°C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the patient samples will not be affected.
10. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.

11. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
12. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
13. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
14. Do not use reagents beyond expiry date as shown on the kit labels.
15. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiterplate readers.
16. 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.
17. Avoid contact with *Stop Solution* containing 0.5 M H_2SO_4. It may cause skin irritation and burns.
18. Some reagents contain Proclin 300, BND and/or MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.
19. TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
20. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
21. For information on hazardous substances included in the kit please refer to Material Safety Data Sheets. Material Safety Data Sheets for this product are available upon request directly from DRG.

4 REAGENTS

4.1 Reagents provided

1. ***Microtiterwells***, 12x8 (break apart) strips, 96 wells;
   Wells coated with anti-L1-CAM antibody (monoclonal).
2. ***Standard (Standard 0-5)***, 6 vials (lyophilized), 1 mL;
   Concentrations: 0 – 8.75 – 35.0 – 70.0 – 140.0 – 280 ng/mL
   See „Preparation of Reagents“;
   Contain non-mercury preservative.
3. ***Control Low & High***, 2 vials (lyophilized), 1.0 mL,
   see „Reagent Preparation“
   For control values and ranges please refer to vial label or QC-Datasheet.
   Contains non-mercury preservative.
4. ***Enzyme Conjugate***, 1 vial, 14 mL, ready to use,
   Anti-L1-CAM antibody, biotinylated;
   Contains non-mercury preservative.
5. ***Enzyme Complex***, 1 vial, 14 mL, ready to use
   contains streptavidin peroxidase,
   Contains non-mercury preservative.
6. ***Substrate Solution***, 1 vial, 14 mL, ready to use,
   Tetramethylbenzidine (TMB).
7. ***Stop Solution***, 1 vial, 14 mL, ready to use,
   contains 0.5M H_2SO_4,
   Avoid contact with the stop solution. It may cause skin irritations and burns.
8. ***Wash Solution***, 1 vial, 30 mL (40X concentrated),
   see „Preparation of Reagents“.

4.2 Materials required but not provided

- A microtiter plate calibrated reader (450 ± 10 nm) (e.g. the DRG Instruments Microtiter Plate Reader).
- Calibrated variable precision micropipettes.
- Absorbent paper.
- Distilled or deionized water
- Timer
- Semi logarithmic graph paper or software for data reduction

4.3 Storage Conditions

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 two months if stored as described above.

4.4 Reagent Preparation

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

Standards

Reconstitute the lyophilized contents of the standard vials with 1.0 mL Aqua dest.

Note: *The reconstituted standards are stable for 2 months at 2 °C to 8 °C.*
For longer storage freeze at -20°C.

Control

Reconstitute the lyophilized content with 1.0 mL Aqua dest. and let stand for 10 minutes in minimum. Mix the control several times before use.

Note: *The reconstituted control is stable for 2 months at 2 °C to 8 °C. For longer storage freeze at -20°C.*

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.

4.5 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 Sheet.

4.6 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.

5 SPECIMEN COLLECTION AND PREPARATION

Serum can be used in this assay.

Do not use haemolytic, icteric or lipaemic specimens.

Please note: Samples containing sodium azide should not be used in the assay.

5.1 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.

5.2 Specimen Storage and Preparation

Specimens should be capped and have immediately to be stored frozen at ≤ -20 °C prior to assaying.

Thawed samples should be inverted several times prior to testing.

5.3 Specimen Dilution

If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with 0.9 % NaCl and reassayed as described in Assay Procedure.

For the calculation of the concentrations this dilution factor has to be taken into account.

Example:

a) dilution 1:5 20 µL serum + 80 µL 0.9 % NaCl (mix thoroughly)

b) dilution 1:10: 10 µL serum + 90 µL 0.9 % NaCl (mix thoroughly)

6 ASSAY PROCEDURE

6.1 General Remarks

- All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- 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.

6.2 Test Procedure

Each run must include a standard curve.

1. Secure the desired number of Microtiter wells in the frame holder.
2. Dispense **50 µL** of each ***Standard, Control*** and **samples** with new disposable tips into appropriate wells.
3. Dispense **100 µL** ***Enzyme Conjugate*** into each well.
4. Incubate for **60 minutes** at room temperature on a shaker with 300 rpm.
5. Briskly shake out the contents of the wells.
   Rinse the wells **5 times** with diluted *Wash Solution* (400 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!
6. Dispense **100 µL** ***Enzyme Complex*** into each well.
7. Incubate for **30 minutes** at room temperature.
8. Briskly shake out the contents of the wells.
   Rinse the wells **5 times** with diluted *Wash Solution* (400 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.
9. Add **100 µL** of ***Substrate Solution*** to each well.
10. Incubate for **15 minutes** at room temperature.
11. Stop the enzymatic reaction by adding **100 µL** of ***Stop Solution*** to each well.
12. Determine the absorbance (OD) of each well at **450 ± 10 nm** with a microtiter plate reader.
    It is recommended that the wells be read **within 10 minutes** after adding the *Stop Solution*.

6.3 Calculation of Results

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Using semi-logarithmic graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted or reported as > 280 ng/mL. For the calculation of the concentrations this dilution factor has to be taken into account.

6.3.1 Example of Typical Standard Curve

The following data is for demonstration only and **cannot** be used in place of data generations at the time of assay.

Standard (ng/mL)	Optical Units (450 nm)
Standard 0 (0)	0.06
Standard 1 (8.75)	0.12
Standard 2 (35.0)	0.46
Standard 3 (70.0)	0.83
Standard 4 (140.0)	1.52
Standard 5 (280.0)	2.66

7 EXPECTED NORMAL VALUES

It is strongly recommended that each laboratory should determine its own normal and abnormal values.

In a study conducted with apparently normal healthy adults, using the DRG L1-CAM ELISA the following values are observed:

Population	Valid N	Range (ng/mL)	Mean (ng/mL)
Males and females	39	≤ 2.7 – 16.9	9.0
Ascetis liquid of females tumour patients	22	3.2 - > 280	71.3

The results alone should not be the only reason for any therapeutic consequences. The results should be correlated to other clinical observations and diagnostic tests.

8 QUALITY CONTROL

Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.

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.

The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate added to the kit. The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results.

It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.

Employ appropriate statistical methods for analysing control values and trends. 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.

9 PERFORMANCE CHARACTERISTICS

9.1 Assay Dynamic Range

The range of the assay is between 2.74 – 280 ng/mL.

9.2 Specificity of Antibodies (Cross Reactivity)

Data will follow.

9.3 Sensitivity

The analytical sensitivity of the DRG ELISA was calculated by adding 2 standard deviations to the mean of 20 replicate analyses of the Zero Standard (S0) and was found to be < 2.74 ng/mL.

9.4 Reproducibility

9.4.1 Intra Assay

The within assay variability is shown below:

Sample	n	Mean (ng/mL)	CV (%)
1	20	15.37	2.6
2	20	74.84	4.3
3	20	131.52	5.5

9.4.2 Inter Assay

The between assay variability is shown below:

Sample	n	Mean (ng/mL)	CV (%)
1	20	18.41	12.2
2	20	63.36	10.9
3	20	113.68	11.5

9.5 Recovery

Samples have been spiked by adding L1-CAM solutions with known concentrations.

The % Recovery has been calculated by multiplication of the ratio of the measurements and the expected values with 100.

		Sample 1	Sample 2	Sample 3
Concentration [ng/mL]		77.02	134.70	54.10
Average Recovery		99.7	97.9	99.9
Range of Recovery [%]	from	98.9	93.9	97.8
	to	100.9	102.0	103.3

9.6 Linearity

		Sample 1	Sample 2	Sample 3
Concentration [ng/mL]		148.00	139.30	42.20
Average Recovery		98.9	96.4	101.1
Range of Recovery [%]	from	96.8	95.3	92.9
	to	102.2	97.9	108.1

10 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.

10.1 Interfering Substances

Haemoglobin (up to 4 mg/mL), Bilirubin (up to 0.5 mg/mL) and Triglyceride (up to 30 mg/mL) have no influence on the assay results.

10.2 Drug Interferences

Until today no substances (drugs) are known to us, which have an influence to the measurement of L1-CAM in a sample.

10.3 High-Dose-Hook Effect

No hook effect was observed in this test up to 690 ng/mL of L1-CAM.

11 LEGAL ASPECTS

11.1 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.

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.

11.2 Therapeutic Consequences

Therapeutic 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.

Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutic consequences be derived.

The test result itself should never be the sole determinant for deriving any therapeutic consequences.

11.3 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.

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Symbols used with DRG ELISAs

Symbol	English	Deutsch	Français	Español	Italiano
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conforme aux normes européennes	Conformidad europea	Conformità europea
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las Instrucciones	Consultare le istruzioni per l'uso
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
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
Σ	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 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
Antiserum	Antiserum	Antiserum	Antisérum	Antisuero	Antisiero
Enzyme Conjugate	Enzyme Conjugate	Enzymkonjugat	Conjugué enzymatique	Conjugado enzimático	Tracciante enzimatico
Enzyme Complex	Enzyme Complex	Enzymkomplex	Complexe enzymatique	Complex enzimático	Complesso 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	Zero Standard	Estándar cero	Standard zero
Standard	Standard	Standard	Standard	Estándar	Standard
Control	Control	Kontrolle	Contrôle	Control	Controllo
Assay Buffer	Assay Buffer	Assaypuffer	Tampon d'essai	Tampón de ensayo	Tampone del test
Wash Solution	Wash Solution	Waschlösung	Solution de lavage	Solución de lavado	Soluzione di lavaggio
1N NaOH	1N NaOH	1N NaOH	1N NaOH	1N NaOH	1N NaOH (idrossido di sodio 1N)
1 N HCl	1 N HCl	1 N HCl	1N HCl	1 N HCl	
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

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Symbol	Portugues	Dansk	Svenska	Ελληνικά
CE	Conformidade com as normas europeias	Europaeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση
	Consulte as instruções de utilização	Se brugsanvisning	Se bruksanvisningen	Εγχειρίδιο χρήστη
IVD	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό
RUO				
REF	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου
LOT	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος
		Indeholder tilstrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις
	Temperatura de conservação	Opbevaringstemperatur	Förvaringstempratur	Θερμοκρασία αποθήκευσης
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης
	Fabricante	Producent	Tillverkare	Κατασκευαστής
Distributed by				
Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο
Volume/No.	Volume/Número	Volumen/antal	Volym/antal	Όγκος/αριθ..
Microtiterwells	Alvéolos de microtitulação	Mikrotiterbrønde	Brunnar i Mikrotiterplatta	Πηγαδάκια Μικροτιτλοδοτήσεως
Antiserum	Anti-soro	Antiserum	Antiserum	Αντιορός
Enzyme Conjugate	Conjugado enzimático	Enzymkonjugat	Enzymkonjugat	Συζευγμένο ενζυμο
Enzyme Complex	Complexo enzimático	Enzymkompleks	Enzymkomplex	Σύμπλοκο ενζύμου
Substrate Solution	Solução de substrato	Substratopløsning	Substratlösning	Διάλυμα υποστρώματος
Stop Solution	Solução de paragem	Stopopløsning	Stopp lösning	Διάλυμα τερματισμού
Zero Standard	Padrão zero	Standard 0	Standard 0	Πρότυπο Μηδέν
Standard	Calibrador	Standard	Standard	Πρότυπα
Control	Controlo	Kontrol	Kontroll	Έλεγχος
Assay Buffer	Tampão de teste	Assay buffer	Assay Buffer	Ρυθμιστικό Διάλυμα Εξέτασης
Wash Solution	Solução de lavagem	Vaskebuffer	Tvätt lösning	Διάλυμα πλύσεως
1N NaOH	1N NaOH	1N NaOH	1N NaOH	1N NaOH
1 N HCl	1 N HCl	1 N HCl	1 N HCl	1 N HCl
Sample Diluent				
Conjugate Diluent				