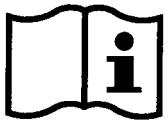
Product information



Userś Manual

Laminin-1 IgG ELISA

Enzyme Immunoassay for the determination of IgG antibodies directed against Laminin-1 in human serum.

REF AE29014

Σ 96

RUO

For Research Use Only – Not for Use in Diagnostic Procedures

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Version
RUO:2021-05-31
TCV

1 INTENDED USE

This testkit is a solid phase enzyme immunoassay employing highly purified native human Laminin-1 for the separate quantitative detection of IgG antibodies against Laminin-1 in human serum.

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2 PRINCIPLE OF THE TEST

Principle of the test

Serum samples diluted 1:101 are incubated in the microplates coated with the specific antigen. The IgG antibodies, if present in the specimen, bind to the antigen. The unbound fraction is washed off in the following step. Afterwards anti-human immunoglobulins conjugated to horseradish peroxidase (conjugate) are incubated and react with the antigen-antibody complex of the samples in the microplates. Unbound conjugate is washed off in the following step. Addition of TMB-substrate generates an enzymatic colorimetric (blue) reaction, which is stopped by diluted acid (color changes to yellow). The intensity of color formation from the chromogen is a function of the amount of conjugate bound to the antigen-antibody complex and this is proportional to the initial concentration of the respective antibodies in the sample.

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3 REAGENTS

TO BE RECONSTITUTED				
Item	Quantity	Cap color	Solution color	Description / Contents
Sample Buffer (5x)	1 x 20ml	White	Yellow	5 x concentrated Tris, sodium chloride (NaCl), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Wash Buffer (50x)	1 X 20ml	White	Green	50 x concentrated Tris, NaCl, Tween 20, sodium azide < 0.1% (preservative)
READY TO USE				
Item	Quantity	Cap color	Solution color	Description / Contents
Negative Control	1 x 1.5ml	Green	Colorless	Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Positive Control	1 x 1.5ml	Red	Yellow	Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Calibrators	6 x 1.5ml	White	Yellow *	Concentration of each calibrator: 0, 3, 10, 30, 100, 300 U/ml. Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Conjugate IgG	1 x 15ml	Blue	Blue	Anti-human immunoglobulins conjugated to horseradish peroxidase, bovine serum albumin (BSA)
TMB Substrate	1 x 15ml	Black	Colorless	Stabilized tetramethylbenzidine and hydrogen peroxide (TMB/H_2O_2)
Stop Solution	1 x 15ml	White	Colorless	1M Hydrochloric Acid
Microtiter plate	12 x 8 well strips	N/A	N/A	With breakaway microwells. Refer to paragraph 1 for coating.
* Color increasing with concentration				
MATERIALS REQUIRED, BUT NOT PROVIDED				
Microtiter plate reader 450 nm reading filter and recommended 620 nm reference filter (600-690 nm). Glass ware (cylinder 100-1000ml), test tubes for dilutions. Vortex mixer, precision pipettes (10, 100, 200, 500, 1000 µl) or adjustable multipipette (100-1000µl). Microplate washing device (300 µl repeating or multichannel pipette or automated system), adsorbent paper. Our tests are designed to be used with purified water according to the definition of the United States Pharmacopeia (USP 26 - NF 21) and the European Pharmacopeia (Eur.Ph. 4th ed.).				

4 STORAGE CONDITIONS

Store all reagents and the microplate at 2-8°C/35-46°F, in their original containers. Once prepared, reconstituted solutions are stable at 2-8°C/35-46°F for 1 month. Reagents and the microplate shall be used within the expiry date indicated on each component, only. Avoid intense exposure of TMB solution to light. Store microplates in designated foil, including the desiccant, and seal tightly.

5 WARNINGS AND PRECAUTIONS

1. **This product is for research use only.** Thus, only staff trained and specially advised in method of ELISA techniques may perform the kit. **Not for use in diagnostic procedures.**
2. This kit contains potentially hazardous components. Though kit reagents are not classified being irritant to eyes and skin we recommend to avoid contact with eyes and skin and wear disposable gloves.
3. Calibrators, Controls, and Buffers contain sodium azide (NaN3) as a preservative. NaN3 may be toxic if ingested or adsorbed by skin or eyes. NaN3 may react with lead or copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build up. Please refer to decontamination procedures as outlined by CDC or other local/national guidelines.
4. Do not smoke, eat or drink when manipulating the kit. Do not pipette by mouth.

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5. All human source material used for some reagents of this kit (controls, standards e.g.) has been tested by approved methods and found negative for HbsAg, Hepatitis C and HIV 1. However, no test can guarantee the absence of viral agents in subh material completely. Thus handle kit controls, standards and samples as if capable of transmitting infectious diseases and according to national requirements.
6. The kit contains material of animal origin as stated in the table of contents, handle according to national requirements.
7. In case that the product information, including the labelling, is defective or incorrect please contact the manufacturer or the supplier of the test kit.
8. Do not mix or substitute Controls, Calibrators, Conjugates or microplates from different lot numbers. This may lead to variations in results.
9. Allow all components to reach room temperature (20-32ºC/68-89.6ºF) before use, mix well and follow the recommended incubation scheme for an optimum performance of the test.
10. Incubation: We recommend test performance at 30ºC/86ºF for automated systems.
11. Never expose components to higher temperature than 37ºC/98.6ºF.
12. Always pipette substrate solution with brand new tips only. Protect this reagent from light. Never pipette conjugate with tips used with other reagents prior.

6 SAMPLE COLLECTION AND PREPARATION

Use preferentially freshly collected serum samples. Blood withdrawal must follow national requirements. Do not use icteric, lipemic, homolysed or bacterially contaminated samples. Sera with particles should be cleared by low-speed centrifugation (<1000 x g). Blood samples should be collected in clean, dry and empty tubes.
After separation, the serum samples should be used during the first 8h, respectively stored tightly closed at 2-8ºC/35-46ºF up to 48h, or frozen at -20ºC/-4ºF for longer periods.

7 PREPARATION OF REAGENTS AND SAMPLES

Dilute concentrated reagents:

Dilute the concentrated sample buffer 1:5 with distilled water (e.g. 20 ml plus 80 ml).

Dilute the concentrated wash buffer 1:50 with distilled water (e.g. 20 ml plus 980 ml).

To avoid mistakes we suggest to mark the cap of the different calibrators.

Samples:

Dilute serum samples 1:101 with sample buffer (1x)

e.g. 1000 µl sample buffer (1x) + 50 µl serum. Mix well !

Washing:

Prepare 20 ml of diluted wash buffer (1x) per 8 wells or 200 ml for 96 wells

e.g. 4 ml concentrate plus 196 ml distilled water.

Automated washing:

Consider excess volumes required for setting up the instrument and dead volume of robot pipette.

Manual washing:

Discard liquid from wells by inverting the plate. Knock the microwell frame with wells downside vigorously on clean adsorbent paper. Pipette 300 µl diluted wash buffer in each well, wait for 20 seconds. Repeat the whole procedure twice again.

Microplates:

Calculate the number of wells required for the test. Remove unused wells from the frame, replace and store in the provided plastic bag, together with desiccant, seal tightly (2-8°C/35-46°F).

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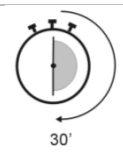
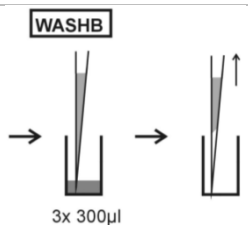
8 ASSAY PROCEDURE

We suggest pipetting calibrators, controls and samples as follows:

	1	2	3	4...
A	Cal A	Cal E	P1	
B	Cal A	Cal E	P1	
C	Cal B	Cal F	P2	
D	Cal B	Cal F	P2	
E	Cal C	PC	P3	
F	Cal C	PC	P3	
G	Cal D	NC	…	
H	Cal D	NC	…	

Cal A: calibrator A
Cal B: calibrator B
Cal C: calibrator C
Cal D: calibrator D
Cal E: calibrator E
Cal F: calibrator F
PC: positive control
NC: negative control
P1: sample 1
P2: sample 2
P3: sample 3

Step	*Description*
1.	Ensure preparations from step 7 above have been carried out prior to pipetting.
2.	Use the following steps in accordance with the intended quantitative interpretation of the results:
	Calibrators, controls, and samples
3.	 Pipette into the designated wells as described in chapter 8 above, 100 µl of: a. Calibrators (CAL.A to CAL.F) for *QUANTITATIVE* and 100 µl of each of the following: • Negative control (NC) and Positive control (PC), and • Diluted sample serum (P1, P2...)
4.	 Incubate for 30 minutes at 20-32°C/68-89.6°F.
5.	 Wash 3x in each case with 300 µl washing buffer (diluted 1:50).

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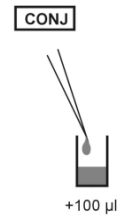
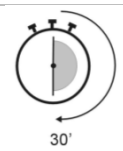
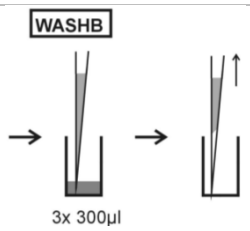
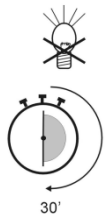
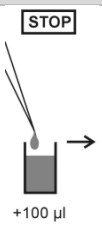
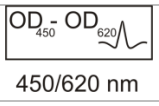
	CONJUGATE	
6.		Pipette 100 µl conjugate into each well.
7.		Incubate for 30 minutes at 20-32°C/68-89.6°F.
8.		Wash 3x in each case with 300 µl washing buffer (diluted 1:50).
	SUBSTRATE	
9.		Pipette 100 µl TMB substrate into each well.
10.		Incubate for 30 minutes at 20-32°C/68-89.6°F, protected from intense light.
	STOP	
11.		Pipette 100 µl stop solution into each well, using the same order as pipetting the substrate.
12.		Incubate 5 minutes minimum.
13.		Agitate plate carefully for 5 sec.
14.		Read absorbance at 450 nm (recommended 450/620 nm) within 30 minutes.

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9 INTERPRETATION

For **quantitative interpretation** establish the standard curve by plotting the **optical density** (**OD) of each calibrator (y-axis)** with respect to the corresponding concentration values in U/ml (x-axis). For best results we recommend log/lin coordinates and 4-Parameter Fit. From the OD of each sample, read the corresponding antibody concentrations expressed in U/ml.

Normal Range	Equivocal Range	Positive Results
< 12 U/ml	12 - 18 U/ml	>18 U/ml

Example of a standard curve

Do not use this example for interpretating results

Calibrators	OD 450/620 nm	CV % (variance)
0 U/ml	0.046	1.5
3 U/ml	0.145	4.4
10 U/ml	0.301	6.8
30 U/ml	0.609	9.3
100 U/ml	1.265	2.2
300 U/ml	2.102	2.8

Example calculation

Sample	Replication (OD)	Mean (OD)	Result (U/ml)
P 01	0.943/0.975	0.959	62.4
P 02	0.618/0.633	0.626	31.0

Samples that are above the highest calibrator value should be reported as > max. They should be diluted accordingly and be re-evaluated, taking the dilution factor into account. Samples lower than the measurement range should be reported as < min.

For lot-specific data please see the attached QC certificate. Medical laboratories should perform in-house quality controls with their own controls and/or pooled sera according to national legislation.

It is recommended that each laboratory works out its own normal values, based on its own technology, controls, equipment and population.

If the control values do not meet the validation criteria, the test is invalid and must be repeated.

The following technical data should be reviewed: expiry dates of the reagents, storage conditions, pipettes, used equipment, photometer, incubation conditions and washing methods.

If the tested samples reveal unusual values or deviations, or if the validation criteria are not met for inexplicable reasons, please contact the IBL-America.

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10 TECHNICAL DATA

Sample material:	Serum
Sample volume:	10 µl of a 1:101 sample dilution with 1x sample buffer
Total incubation period:	90 minutes at 20-32°C/68-89.6°F.
Measurement range:	0-300 U/ml
Analytical sensitivity:	1.0 U/ml
Storage:	at 2-8°C/35-46°F in original bottles only.
Number of determinations:	96 tests

11 LITERATURE

Burgeson RE, Chiquet M, Deutzmann R, Ekblom P, Engel J, Kleinmann H, Martin GR, Meneguzzi G, Paulsson M, Sanes J. A new nomenclature for the laminins. Matrix Biol 1994; 14: 209-211.

Matalon ST, Blank M, Matsuura E, Inagaki J, Nomizu M, Levi Y, Koike T, Shere Y, Ornoy A, Shoenfeld Y. Immunizatuion of naïve mice with mouse laminin-1 affected pregnancy putcome in a mouse model. Am J Reprod Immunol 2003; 50: 159-165.

Inagaki J, Matsuura E, Nomizu M, Suguira-Ogasawara M, Datano K, Kaihara K, Kobayashi K, Yasuda T, Aoki K. IgG anti-laminin-1 autoantibody and recurrent miscarriages. Am J Reprod Immunol 2001; 45: 232-238.

Inagaki J, Suguira-Ogasawara M, Nomizu M, Levi Y, Koike T, Shere Y, Ornoy A, Shoenfeld Y, Aoki K, Matsuura E. An association of IgG anti-laminin-1 autoantibodies with endometriosis in infertile patients. Human Reprod. 2003; 18: 544-549.

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SYMBOLS USED WITH IBL-AMERICA 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-Konfirmitäts-kennzeichnung	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

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