研究用試薬

CXCL13 ELISA
Test instruction

ORDER NO.	ANTIGEN	SUBSTRATE	FORMAT
EQ 6811-9601-L	CXCL13	Ab-coated microplate wells	96 x 01 (96)

Indication: This ELISA test kit provides quantitative in vitro determination of CXCL13 in human cerebrospinal fluid (CSF) of patients with suspected encephalitis, especially in cases of suspected borreliosis with involvement of the central nervous system (neuroborreliosis).

Application: The CXCL13 ELISA determines the chemokine CXCL13 in the CSF. Increased CXCL13 levels in CSF are frequently detectable in acute neuroborreliosis, but also in other inflammatory diseases of the CNS such as neurosyphilis, HIV meningitis, streptococcus infections, toxoplasmosis and multiple sclerosis. With successful therapy, the CXCL13 concentration in the CSF sinks rapidly, which can be considered as an indicator of successful treatment.

Principles of the test: In the first analysis step, the calibrators and patient samples are diluted with biotin-labelled anti-CXCL13 and added to microplate wells coated with monoclonal anti-CXCL13 antibodies. In this process CXCL13 is bound in a complex. In a second incubation, this complex is labelled with peroxidase-labelled streptavidin. In a third incubation using the substrate tetra-methylbenzidine (TMB) the bound peroxidase promotes a colour reaction. The colour intensity is proportional to the CXCL13 concentration in the sample.

Contents of the test kit:

Component	Colour	Format	Symbol
1. Antibody-coated microplate wells 12 microplate strips each containing 8 individual break-off wells in a frame, ready for use	---	12 x 8	STRIPS
2. Calibrator 1, CXCL13, ready for use	colourless	1 x 1 ml	CAL 1
3. Calibrator 2, CXCL13, lyophilised	white	1 x 500 µl	CAL 2
4. Calibrator 3, CXCL13, lyophilised	white	1 x 500 µl	CAL 3
5. Calibrator 4, CXCL13, lyophilised	white	1 x 500 µl	CAL 4
6. Calibrator 5, CXCL13, lyophilised	white	1 x 500 µl	CAL 5
7. Calibrator 6, CXCL13, lyophilised	white	1 x 500 µl	CAL 6
8. Control 1, CXCL13, lyophilised	white	1 x 500 µl	CONTROL 1
9. Control 2, CXCL13, lyophilised	white	1 x 500 µl	CONTROL 2
10. Biotin, biotin-labelled CXCL13 detection antibody, ready for use	green	1 x 7 ml	BIOTIN
11. Enzyme conjugate peroxidase-labelled streptavidin, ready for use	blue	1 x 12 ml	CONJUGATE
12. Wash buffer 10x concentrate	colourless	1 x 100 ml	WASH BUFFER 10x
13. Chromogen/substrate solution TMB/H_2O_2, ready for use	colourless	1 x 12 ml	SUBSTRATE
14. Stop solution 0.5 M sulphuric acid, ready for use	colourless	1 x 12 ml	STOP SOLUTION
15. Quality control certificate	---	1 protocol	
16. Test instruction	---	1 booklet	
17. Protective foil	---	3 pieces	FOIL

LOT Lot description
IVD In vitro diagnostic medical device
CE
Storage temperature
Unopened usable until

Preparation and stability of the reagents

Note: All reagents must be brought to room temperature (+18°C to +25°C) approx. 30 minutes before use. After first use, the reagents are stable until the indicated expiry date if stored at +2°C to +8°C and protected from contamination, unless stated otherwise below.

- **Coated wells:** Ready for use. Tear open the resealable protective wrapping of the microplate at the recesses above the grip seam. Do not open until the microplate has reached room temperature to prevent the individual strips from moistening. Immediately replace the remaining wells of a partly used microplate in the protective wrapping and tightly seal with the integrated grip seam (Do not remove the desiccant bag).
  Once the protective wrapping has been opened for the first time, the wells coated with antibodies can be stored in a dry place and at a temperature between +2°C and +8°C for 4 months.

- **Calibrators and controls:** Reconstitute calibrators 2 to 6 and controls with 500 µl deionised or distilled water approximately 10 minutes before use and mix thoroughly upside down. Before use, confirm that the lyophilisate is completely dissolved in the water. The reconstituted calibrators and controls must be frozen at -20°C immediately after use. The calibrators and controls can be frozen and thawed up to three times. Longer residence times at room temperature must be avoided at all costs.

- **Biotin:** Ready for use. Mix thoroughly before use.

- **Enzyme conjugate:** Ready for use. Mix thoroughly before use.

- **Wash buffer:** The wash buffer is a 10x concentrate. If crystallisation occurs in the concentrated buffer, warm it to +37°C and mix well before diluting. The quantity required should be removed from the bottle using a clean pipette and diluted with deionised or distilled water (1 part reagent plus 9 parts distilled water).
  For example: For 1 microplate strip, 5 ml concentrate plus 45 ml water.
  The working strength wash buffer is stable for 4 weeks when stored at +2°C to +8°C and handled properly.

- **Chromogen/substrate solution:** Ready for use. Close the bottle immediately after use, as the contents are sensitive to light ✳. The chromogen/substrate solution must be clear on use. Do not use the solution if it is blue coloured.

- **Stop solution:** Ready for use.

Storage and stability: The test kit has to be stored at a temperature between +2°C to +8°C. Do not freeze. Unopened, all test kit components are stable until the indicated expiry date.

Waste disposal: Patient samples, calibrators, controls and incubated microplate strips should be handled as infectious waste. All reagents must be disposed of in accordance with local disposal regulations.

Warning: Some of the reagents contain preserving agents in a non-declarable concentration. Avoid skin contact.

Preparation and stability of the patient samples

Samples: Cerebrospinal fluid (CSF).

Advice on sample storage: The recommended storage time of patient samples to be investigated is up to 14 days at +2°C to +8°C. Only for long-time storage we recommend a storage temperature of -20°C.

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Incubation

(Partly) manual test performance

Sample incubation: (1st step)

Pipette **50 µl** of the calibrators, controls and undiluted patient samples and **50 µl** of biotin into each of the reagent wells.
Cover the reagent wells with the provided protective foil and incubate for **180 minutes** on a **microplate shaker (400 rpm)** at room temperature (+18°C to +25°C).

Washing:

Manual: Remove the protective foil, empty the wells and subsequently wash 3 times using 300 µl of working strength wash buffer for each wash.
Automatic: Remove the protective foil and wash the reagent wells 3 times with 450 µl of working strength wash buffer (program setting: e.g. TECAN Columbus Washer "Overflow Mode").

Leave the wash buffer in each well for 30 to 60 seconds per washing cycle, then empty the wells. After washing (manual and automated tests), thoroughly dispose of all liquid from the microplate by tapping it on absorbent paper with the openings facing downwards to remove all residual wash buffer.

Note: Residual liquid (>10 µl) in the reagent wells after washing can interfere with the substrate and lead to false low extinction readings.
Insufficient washing (e.g. less than 3 wash cycles, too small wash buffer volumes, or too short residence times) can lead to false high extinction readings.
Free positions on the microplate strip should be filled with blank wells of the same plate format as that of the parameter to be investigated.

Enzyme conjugate incubation: (2nd step)

Pipette **100 µl** of enzyme conjugate (streptavidin-peroxidase) into each of the microplate wells, cover with the enclosed protective foil and incubate for **30 minutes** on a **microplate shaker (400 rpm)** at room temperature (+18°C to +25°C).

Washing:

Empty the wells. Wash as described above.

Substrate incubation: (3rd step)

Pipette **100 µl** of chromogen/substrate solution into each of the microplate wells.
Incubate for **15 minutes** at room temperature (+18°C to +25°C), protect from direct sunlight.

Stopping the reaction:

Pipette **100 µl** of stop solution into each of the microplate wells in the same order and at the same speed as the chromogen/substrate solution was introduced.

Measurement:

Photometric measurement of the colour intensity should be made at a **wavelength of 450 nm** and a reference wavelength between 620 nm and 650 nm **within 30 minutes of adding the stop solution**. Prior to measuring, slightly shake the microplate to ensure a homogeneous distribution of the solution.

Test performance using fully automated analysis devices

Sample dilution and test performance are carried out fully automatically using an analysis device. The incubation conditions programmed in the respective software authorised by EUROIMMUN may deviate slightly from the specifications given in the ELISA test instruction. However, these conditions were validated in respect of the combination of the EUROIMMUN Analyzer I, Analyzer I-2P or the DSX from Dynex and this EUROIMMUN ELISA. Validation documents are available on enquiry.
Automated test performance using other fully automated, open-system analysis devices is possible. However, the combination should be validated by the user.

Pipetting protocol

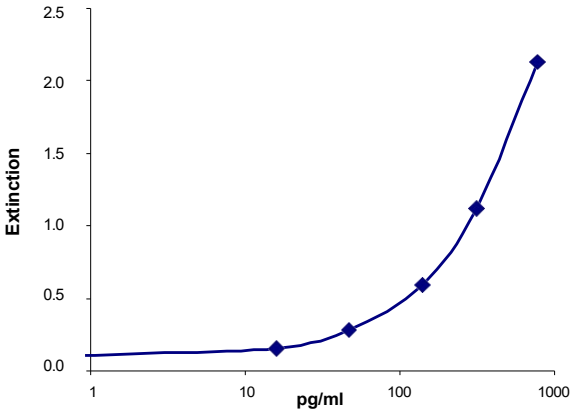
	1	2	3	4	5	6	7	8	9	10	11	12
A	C 1	P 1	P 9									
B	C 2	P 2	P 10									
C	C 3	P 3	P 11									
D	C 4	P 4	P 12									
E	C 5	P 5	P 13									
F	C 6	P 6	P 14									
G	Co 1	P 7	P 15									
H	Co 2	P 8	P 16									

The pipetting protocol for microtiter strips 1 to 3 is an example for the **quantitative analysis** of 16 patient samples (P 1 to P 16).

The calibrators (C 1 to C 6), the controls (Co 1, Co 2), and the patient samples have each been incubated in one well. The reliability of the ELISA test can be improved by duplicate determinations for each sample.
The controls serve as internal controls for the reliability of the test procedure. They should be assayed with each test run.

Calculation of results

Quantitative: The standard curve from which the concentration of CXCL13 in the unknown patient samples can be taken is obtained by plotting of the extinction readings measured for the 6 calibration sera against the corresponding units (linear/log). For computer-aided calculation of the standard curve, the evaluation procedures “5-PL” (5-parameter logistic), “4-PL” (4-parameter logistic), “Cubic spline” or “Akima” shall be selected. For correct logarithmic representation it might be necessary to set the concentration of calibrator 1 from 0 pg/ml to e.g. 0.1 pg/ml. The following plot is an example of a typical calibration curve. Please do not use this curve for the determination of concentrations in patient samples.



If the extinction for a patient sample lies above the extinction of calibrator 6, it is recommended that the sample be retested at an initial dilution of e.g. 1:10 in calibrator 1.
Note: The result in pg/ml read from the calibration curve for this sample must be multiplied by a factor of 10.

For duplicate determinations the mean of the two values should be taken. If the two values deviate substantially from one another, EUROIMMUN recommends retesting the samples.

Therapeutic decisions should not be made on the basis of results from this test, but only under consideration of clinical findings and further diagnostic values.

Test characteristics

Calibration: The exact concentrations of the calibrators and the acceptance ranges of the controls are lot-dependent and given on the quality control certificate enclosed with this test instruction. For every group of tests performed, the concentrations of the controls must lie within the limits stated for the relevant test kit lot. If the values specified for the controls are not achieved, the test results may be inaccurate and the test should be repeated.

Antibodies: The reagent wells are coated with a monoclonal anti-CXCL13 antibody.

Detection limit: The lower detection limit is defined as the mean value of an analyte-free sample plus three times the standard deviation and is the smallest detectable CXCL13 concentration. The lower detection limit of the CXCL13 ELISA is 4.0 pg/ml (average value). The functional sensitivity defined as the lowest concentration of a sample with a CV <20% was found to be 10.8 pg/ml.

Cross reactivity: This ELISA detects CXCL13 specifically. Undesired cross reactions with other chemokines (e.g. CXCL2, CXCL8, CXCL10) could not be observed.

Interference: Contamination with blood up to a concentration of 10% (v/v) did not cause interference with the ELISA. Red tint of the sample indicates significant contamination with blood. The sample should not be used.

High-dose hook effect: No high-dose hook effect was observed up to a concentration of 500,000 pg/ml.

Reproducibility: The reproducibility of the test was investigated by determining the intra- and inter-assay coefficients of variation (CV) using 3 CSF samples. The intra-assay CVs are based on 20 determinations and the inter-assay CVs on triplicates performed in 10 different test runs. The inter-lot CVs are based on duplicates in 3 different test runs performed for each of 3 kit lots.

Intra-assay variation, n = 20		
Sample	**Mean value (pg/ml)**	**CV (%)**
1	22	4.6
2	40	3.0
3	398	2.6

Inter-assay variation, n = 10 x 3		
Sample	**Mean value (pg/ml)**	**CV (%)**
4	21	9.8
5	121	5.7
6	297	3.8

Inter-lot variation, n = 3 x 3 x 2		
Sample	**Mean value (pg/ml)**	**CV (%)**
7	31	7.4
8	84	7.5
9	377	2.7

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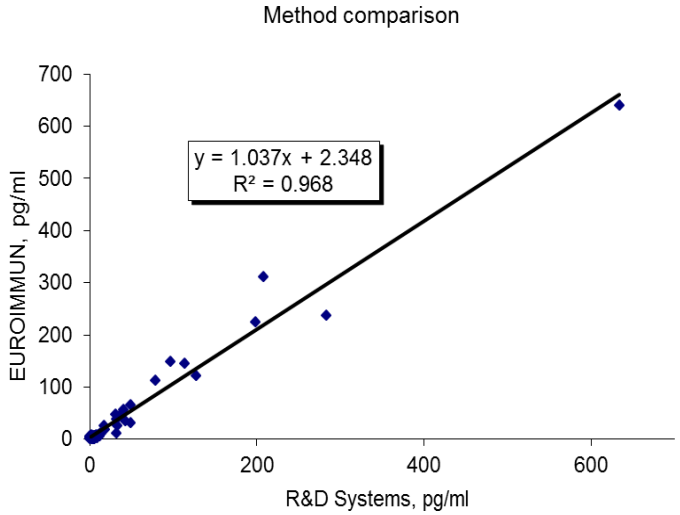
Linearity: The linearity of the test was investigated by diluting three samples in four steps up to 1:16 with sample buffer. The recovery of the expected concentrations ranged from 95% to 117% with a mean correlation coefficient of r = 0.999.

Sample	Dilution (decimal)	Measured (pg/ml)	Recovery (%)
1	1:1	404	
	1:2	192	95
	1:4	99	98
	1:8	50	98
	1:16	28	110
2	1:1	215	
	1:2	103	96
	1:4	56	104
	1:8	31	115
	1:16	15	112
3	1:1	205	
	1:2	101	99
	1:4	54	104
	1:8	30	116
	1:16	15	117

Method comparison: The EUROIMMUN ELISA was compared with one commercially available ELISA of another manufacturer and showed the following correlation:

R&D Quantikine, CXCL13-ELISA	EUROIMMUN = 1.037 x R&D + 2.3 pg/ml; n = 57; r^2 = 0.968



Expected values: 289 CSF samples of patients with unknown anamnesis have been investigated (no known cases of neuroborreliosis). In 90% of these patients, CXCL13 levels in the CSF were <20 pg/ml.

In cases of proven neuroborreliosis (n = 12), CXCL 13 concentrations were >100 pg/ml, frequently there have been values measured outside the calibration curve (>500 pg/ml). Strongly increased CXCL13 levels in CSF can also be observed in neurosyphilis (also Spirochaeta infection) or in chronic lymphatic leukaemia (CLL).

Therefore, we propose evaluating CXCL13 concentrations in CSF as follows:

Standard range:	<20 pg/ml - Exclusion of neuroborreliosis
Borderline range:	20 to 30 pg/ml
Increased:	30 to 100 pg/ml
Strongly increased:	>100 pg/ml - Suspected acute neuroborreliosis if corresponding symptoms are present.

Every laboratory should use their own normal values established under specific ambient conditions.
In recent literature references, for instance, CXCL13 results >250 pg/ml were considered as indicative of acute neuroborreliosis.

Clinical significance

The chemotactic cytokine (chemokine) CXCL13 is a cellular messenger which is produced by monocytes, macrophages and dendritic cells. It is an important chemoattractant for lymphocytes in the cerebrospinal fluid (CSF).

The determination of CXCL13 in CSF is particularly relevant for the diagnosis of neuroborreliosis.

Diagnosis of acute neuroborreliosis has up until now been based on the typical clinical picture (meningitis, meningoradiculitis, neurological deficits), the detection of an inflammatory CSF syndrome (for example pleocytosis, blood-CSF barrier dysfunction) and the detection of intrathecal synthesis of Borrelia-specific antibodies. The antibody detection does not, however, provide information on the activity of the infection. Unlike in serum, the detection of IgM in CSF is not an indicator of acute infection. The classic changeover in immunoglobulin classes is also not observed in CSF. Furthermore, persistence of Borrelia-specific IgG and/or IgM antibodies despite suitable therapy hinders the reliable differentiation of past and active infections.

Studies described in the literature have demonstrated that CXCL13 is a promising marker for identifying acute neuroborreliosis and assessing therapy success. CXC13 can be detected in high concentrations soon after the start of the illness, sometimes even before the production of antibodies. In contrast, the CXCL13 concentration in the serum is not elevated. With successful therapy the CXCL13 concentration in the CSF sinks rapidly, so that CXCL13 analysis is also highly suited to monitoring the disease course.

Increased CXCL13 levels in CSF can also occur in other diseases such as neurosyphilis, HIV meningitis, streptococcus infections, toxoplasmosis and multiple sclerosis.

Borrelia burgdorferi s.l. (senso lato), a bacterium of the Spirochaetaceae family, is the causative agent of Lyme borreliosis. The pathogen is transmitted to people via tick bites. Of around 30 types of ticks that bite humans, Ixodes ricinus is the most common in Europe. It transmits Anaplasma phagocytophilum and TBE virus in addition to Borrelia burgdorferi. Ticks of the type I. ricinus go through three developmental stages: larval, nymph and adult (female and male). The density of I. ricinus ticks is sometimes very high; in some regions it is more than 300 ticks per 100 m^2.

Lyme borreliosis is the most frequent tick-transmitted disease in the northern hemisphere. In Europe, particularly in central Europe and Scandinavia, 155 people per 100,000 are affected by Lyme borreliosis caused by the pathogens Borrelia burgdorferi sensu stricto, Borrelia afzelii and Borrelia garinii. The seroprevalence of antibodies against Borrelia burgdorferi s.l. in the general population in Germany and other central European countries is around 8% (for IgG). In highly endemic regions it is much higher, lying between 18% and 52%. In eastern Asia, for example in an endemic region of China, it is 26%. Borrelia antibodies are exhibited by 40% of forestry workers and 50% of professional hunters. A Borrelia burgdorferi infection can manifest with dermatological, neurological, ophthalmological, rheumatological or internal symptoms. The clinical expression of borreliosis is divided into three stages:

Stage I: The typical primary manifestation of a Borrelia burgdorferi infection is erythema migrans (80%), a reddening of the skin which appears around the area of the tick bite and spreads in a circular manner. The erythema is accompanied by influenza-like general symptoms with fever, chills, headache and vomiting. Lymphadenopathies are observed in a few cases (lymphadenosis cutis benigna). The most common types of erythema-free borreliosis (20%) are the neurological, arthromyalgic, flu-like and cardiovascular forms, borreliosis with hepatitis and regional lymphadenitis, and combinations of any of these forms. Stage I can result in spontaneous healing or can develop into generalised borreliosis. The transition phase is generally symptom-free.

Stage II: A variety of symptoms can develop several weeks to months after the tick bite. In the foreground of these are neurological manifestations: meningitis, encephalitis, asymmetric polyneuritis, cranial nerve pareses, Bannwarth´s lymphocytic meningoradiculitis. One of the most frequent neurological manifestations of borreliosis in children is peripheral facial paralysis. The paralysis of the facial nerve typically affects only one side initially and after a few weeks the other side. Arthritides, particularly of the knee joints, and non-localised bone, joint and muscle pains are also frequently observed. Cardiological manifestations such as myocarditis or pericarditis are, in contrast, rarer.

Stage III: The typical manifestations of a Borrelia burgdorferi infection in stage III are chronic relapsing erosive arthritis, acrodermatitis chronica atrophicans and progressive encephalomyelitis, which can proceed in a similar way to multiple sclerosis. Without treatment, the tertiary stage can develop over a period of years to decades after the original infection.

Literature references

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