EPITOPE DIAGNOSTICS, INC.

EDI™ Anti-Infliximab CLIA Kit

Chemiluminescence Immunoassay (CLIA) for the quantitative measurement of Anti-Infliximab in Serum.

REF CL0010R RUO 50, 100, 250

INTENDED USE

This Chemiluminescence Immunoassay (CLIA) kit is intended for the quantitative determination of Anti-Infliximab levels in serumusing the ECL100 or ECL25 Immunoassay analyzer. The test is used as an aid in monitoring dosing during Infliximab therapy.

For Research Use only

SUMMARY OF PHYSIOLOGY

Infliximab is a monoclonal antibody used as a drug treatment to decrease or prevent the inflammatory effects of tumor necrosis factor-alpha, or TNF-alpha, in certain patients who have Rheumatoid Arthritis, Crohn's Disease, Ankylosing Spondylitis, Psoriatic Arthritis, Plaque Psoriasis, or Ulcerative Colitis[1,2]. While undergoing treatment, some patients will develop antidrug antibodies that can bind to Infliximab, neutralizing its therapeutic effects[3]. Increased concentration of anti-Infliximab antibodies can lower the drug concentration in the body by increasing drug clearance, decrease the likelihood of reaching and sustaining remission, and potentially cause infusion reactions[3,4]. Measurement of anti-Infliximab antibodies can provide more insight into how the body is reacting to Infliximab, and how to move forward with the treatment.

ASSAY PRINCIPLE

This CLIA is designed, developed, and produced for the quantitative measurement of Infliximab in serum samples. The assay utilizes a two-site "sandwich" technique with two antibodiesthat bind to different epitopesof Anti-Infliximab.

Assay calibrators, controls, or patient samples are added directly to a reaction vessel containing streptavidin coated magnetic particles. Anacridinium ester antibody and a biotin antibodyare added. The magnetic particles capture the biotin antibody as well as an immuno complex in the form of "magnetic particles – biotin Infliximab antibody –Anti-Infliximab– acridinium ester Infliximab antibody".

The materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Then, the trigger solution is added to the reaction vessel and light generated by the reaction is measured with the ECL100 or ECL25 analyzer. The relative light units (RLU) are proportional to the concentration of Infliximab in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve and reported in serum Infliximab concentration.

REAGENTS: PREPARATION AND STORAGE

This test kit must be stored at 2 – 8°C upon receipt. For the expiration date of the kit refer to the label on the kit box. All components are stable until this expiration date. Reagents from different kit lot numbers should not be combined or interchanged.

Standard Batch Quantity: 100/kit

1. Anti-Infliximab Magnetic Particle Solution (L0686)

Qty:	1 x 2.0 mL (50/kit), 1 x 2.8 mL (100/kit), 1 x 7.3 mL (250/kit)
Storage:	2 – 8°C
Preparation:	Ready to Use

2. Biotin Infliximab Antibody (L0687)

Qty:	1 x 3.5 mL (50/kit),1 x 6.0 mL (100/kit), 1 x 13.5 mL (250/kit)
Storage:	2 – 8°C
Preparation:	Ready to Use

3. Acridinium Ester Infliximab Antibody (L0688)

Qty:	1 x 2.3 mL (50/kit), 1 x 3.5 mL (100/kit), 1 x 7.3 mL (250/kit)
Storage:	2 – 8°C
Preparation:	Ready to Use

4. Anti-Infliximab Calibrators (L0689 – L0690)

Liquid Anti-Infliximabin a BSA-based matrix with an azide preservative. Refer to vials for exact concentration.

Qty:	2 x vials of 0.5 mL each
Storage:	2 – 8°C
Preparation:	0.5 mL of Calibrators, mix by inversions or gentle vortexing. Make sure that Calibrators are well mixed before use.

5. Anti-Infliximab Controls (L0691 – L0692)

Liquid Anti-Infliximabin a BSA-based matrix with an azide preservative. Refer to vials for exact concentration.

Qty:	2 x vials of 0.5 mL each
Storage:	2 – 8°C
Preparation:	0.5 mL of Controls, mix by inversions or gentle vortexing. Make sure that Controls are well mixed before use.

SAFETY PRECAUTIONS

The reagents must be used in a professional laboratory environment and are for in vitro diagnostic use. Source material which contains reagents of bovine serum albumin was derived in the contiguous 48 United States. It was obtained only from healthy donor animals maintained under veterinary supervision and found free of contagious diseases. Wear gloves while performing this assay and handle these reagents as if they were potentially infectious. Avoid contact with reagents containing hydrogen peroxide. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes. On contact, flush with copious amounts of water for at least 15 minutes. Use Good Laboratory Practices.

MATERIALS REQUIRED BUT NOT PROVIDED

The instrument only uses materials supplied by Epitope Diagnostics, Inc. When materials available from third-party suppliers are used, Epitope Diagnostics, Inc. takes no responsibility for the validity of results obtained. Material is available for purchase from Epitope Diagnostics, Inc. Please contact your distributor for more information.

1. ECL100 Immunoassay Analyzer or ECL25 Immunoassay Analyzer
2. CL011 Cuvettes (for ECL100) or CL010 Cuvettes (for ECL25)
3. Wash Reagent (P-594)
4. Trigger Solutions A and B (P-595)

SPECIMEN COLLECTION AND PREPARATION

Only 15 µL of human serum or plasma sample is required for Anti-Infliximab measurement in singlet. Samples should not be taken from patients taking biotin-containing multivitamins or dietary supplements at least 48 hours prior to specimen collection. Whole blood should be collected and must be allowed to clot for minimum 30 minutes at room temperature before the serum is separated by centrifugation (850 – 1500 RPM for 10 minutes). The serum should be separated from the clot within three hours of blood collection and transferred to a clean test tube. Serum samples should be stored at 15-25°C for three days, 2-8°C for five days, and –20°C or below for three months. Avoid more than three freeze-thaw cycles of specimen. It is necessary to take care in the sample collection procedure to avoid hemolysis.

Some substances in the samples will interfere with the test results. The common interfering substances and maximum allowable concentrations are as follows:

- bilirubin 60 mg/dL
- triglycerides 1500 mg/dL
- hemoglobin 25 mg/dL
- biotin 200 nmol/L
- For patients receiving high-dose biotin therapy (5 mg/ day), samples must be collected 8 hours after takingthe last dose of biotin

A single assay of this item requires 15 µL sample. This quantity does not include the deadvolume in the sample container, the capacity required for retesting, and other measurement items. For the definition of the minimum required sample size, refer to the equipment manual.

CALIBRATION

An active calibration curve is required for all tests. For the assay, calibration is required for the first-time use of a reagent lot and is valid for 28 days. However, we recommend calibration every 14 days after initial calibration or when either kit control is out of range.

QUALITY CONTROL

The characteristics of patient samples are simulated through controls and are critical to validate the performance of CLIA assays due to the random-access format. Use of controls is left to the discretion of the user, based on good laboratory practices, requirements, and applicable laws. We suggest performing a control test once every day. Quality control results that do not fall within acceptable ranges may indicate invalid test results.

ASSAY PROCEDURE

1. Reagents from different kit lot numbers should not be combined or interchanged. Make sure that there are no air bubbles in any reagents, calibrator, and control vials.

2. Reagent Preparation

2.1 Remove reagent cartridges from packaging and replace the solid caps with the provided soft caps for ECL100. For ECL25, carefully remove the aluminum foil seal on each container on the cartridges.

2.2 For the ECL100, take out the Magnetic Particle bottle and make sure to roll between hands and gently but thoroughly mix until the magnetic particle solution is homogenous. The solution should be uniform with no clumps of magnetic particles visible; this step is vital for assay performance.

- Note: For ECL100, if the Magnetic Particle Solution volume is over 3 mL, it will be provided in a glass bottle. It will need to be transferred from the glass bottle to the plastic vial in the cartridge with the rest of the reagents. Make sure the Magnetic Particle Solution is mixed well before transferring.

2.3 For ECL25, mix the magnetic beads by moving back and forth the bottom part of the cartridge at upright position. Make sure to look inside the cartridge until the solution is uniform with no clumps of magnetic particles visible and no air bubbles. Recap the bottle. Open the top soft cap of all reagent bottles, leaving only the hollow soft rubber.

2.4 The reagents are now ready to be loaded into the ECL100 or ECL25 for calibration.

3. Assay Program

The following table illustrates the protocol used by the ECL100 or ECL25 for instrument operation.

Component	Quality Control Hole (µL)	Sample Hole (µL)
Anti-Infliximab Calibrators (L0689-L0690)	15	-
Samples	-	15
Biotin Infliximab Antibody (L0687)	50	50
Anti-Infliximab Magnetic Particle Solution (L0686)	25	25
Incubation Period		
Wash the reaction cuvette 3 times with wash reagent.		
Acridinium Ester Infliximab Antibody (L0688)	25	25
Incubation Period		
Wash the reaction cuvette 3 times with wash reagent.		
Trigger Solution A (P-595)	200	200
Trigger Solution B (P-595)	200	200

The total incubation time is less than 30 minutes.

INTERPRETATION OF RESULTS

The chemiluminescence analyzer calculates the concentration values of the sample and the control by a standard curve (fitting method: four parameters or point-to-point) and the measured RLU. Values are compared with the range of the marked value. If it exceeds the indicated quality control range, it indicates that the test is unqualified and needs to be re-tested.

Due to methodological differences or antibody/antigen specificity, there may be deviations between the test results of reagents from different manufacturers. Therefore, direct comparisons should not be made to avoid false interpretation.

EXPECTED VALUES

Anti-Infliximab concentrations were measured in serum samples collected from 125 apparently healthy adults using the EDI™ Anti-Infliximab CLIA Kit. The observed range of Anti-Infliximabis summarized in the table below.

	Anti-Infliximab Concentration (ng/mL)
Normal	0.00 – 20.00
Anti-Infliximab Antibodies Present	> 20.00

It is highly recommended that each laboratory should establish their own normal range for Infliximab based on local populations.

LIMITATIONS OF THE PROCEDURE

1. This product is for use on the ECL100 or ECL25Immunoanalyzer only. Refer to the appropriate system manuals and/or Help system for a specific description of installation, start-up, operation, system performance, instructions, calibration, precautions, hazards, maintenance, and troubleshooting.
2. Reagents from different lots cannot be mixed.
3. Test results from this product should not be the sole basis for clinical diagnosis.
4. If the test sample result is higher than the upper limit of the calibration curve, it is recommended to re-measure after dilution according to a certain ratio. The measurement result is recalculated according to the dilution ratio to ensure the accuracy of the result.
5. When the sample concentration of Anti-Infliximab is lower than the detection lower limit, the test result will be reported as < 1.03 ng/mL. When the sample concentration is higher than the detection upper limit, it will be reported as > 6250.00 ng/mL.

PERFORMANCE CHARACTERISTICS

Hook Effect

The assay shows no hook effect up to 1000000.00 ng/mL.

Limit of Blank

The limit of blank (LoB) was determined by 60 replicates in three assays of calibrator matrix to be 0.41 ng/mL.

Limit of Detection

The limit of detection (LoD) was determined by 60 replicates in three assays of low-level samples to be 1.03 ng/mL.

Limit of Quantification

The limit of quantification (LoQ) was determined by 60 replicates in three assays of low-level samples to be 1.65 ng/mL.

Linearity

Linearity was determined by two assays with a diluted standard of high Anti-Infliximab concentration. In each assay, the average of two replicates of each of the diluted samples is used for a correlation analysis against calculated theoretical values. The linearity of this test is up to 6250.00 ng/mL.

Standard	Average Concentration (ng/mL)	Theoretical Concentration (ng/mL)	Linear Recovery (%)	R^2
1	0.00	0.00	-	0.999
2	35.09	31.25	112	
3	69.59	62.50	111	
4	137.20	125.00	110	
5	260.70	250.00	104	
6	1651.50	1562.50	106	
7	3365.50	3125.00	108	
8	6853.50	6250.00	110	

Intra-assay Precision

Precision was determined by measuring eight replicates of three specimens. The results are as follows:

Sample	Average Concentration (ng/mL)	SD	CV (%)
1	10.63	0.28	2.7
2	253.68	3.78	1.5
3	6112.09	332.73	5.4

Inter-assay Reproducibility

Reproducibility was determined by measuring three specimens in twenty-four replicates over the run of three assays. The results are summarized below:

Sample	Average Concentration (ng/mL)	SD	CV (%)
1	11.12	1.09	9.8
2	246.48	11.58	4.7
3	6140.50	403.40	6.6

Cross Reactivity

Cross-reactivity was assessed by analyzing several specimens containing several analytes at elevated concentrations. The results are summarized below:

Analytes	Theoretical Concentration	Measured Concentration (ng/mL)
Osteocalcin	60.54 ng/mL	1.92
Chromogranin A	13985.70 ng/mL	1.35
Infliximab Antibody	57.50 ng/mL	1.09
Anti-Tg Antibody	1076.35 IU/mL	1.35
tTG-IgA Antibody	66.09 U/mL	0.73
DGP-IgA Antibody	60.00 U/mL	1.37

Interference

Bilirubin, hemoglobin, and Intralipid were tested as potential interferents to Anti-Infliximab. Randomly selected samples were spiked with the potential interferents at the concentrations listed in the table below:

Interferent (Concentration tested, mg/mL)		Test (ng/mL)	Control (ng/mL)	Bias (d_{obs}, %)
Bilirubin	0.005	10.28	11.90	-13.6
		454.30	423.00	7.4
	0.01	10.91	11.90	-8.3
		430.65	423.00	1.8
	0.02	10.33	11.90	-13.2
		434.65	423.00	2.8
Hemoglobin	0.0625	9.36	9.35	0.2
		434.00	422.00	2.8
	0.125	9.22	9.35	-1.3
		403.05	422.00	-4.5
	0.25	7.84	9.35	-16.1
		404.65	422.00	-4.1
Intralipid	1.0	12.70	14.22	-10.7
		471.15	475.25	-0.9
	5.0	12.17	14.22	-14.4
		450.30	475.25	-5.2
	10.0	15.93	14.22	12.0
		467.15	475.25	-1.7

WARRANTY

This product is warranted to perform as described in its labeling and literature when used in accordance with all instructions. Epitope Diagnostics, Inc. DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, and in no event shall Epitope Diagnostics, Inc. be liable for consequential damages. Replacement of the product or refund of the purchase price is the exclusive remedy for the purchaser. This warranty gives you specific legal rights and you may have other rights, which vary from state to state.

REFERENCES

1. van Hogezand RA, Verspaget HW. The future role of anti-tumour necrosis factor-alpha products in the treatment of Crohn's disease. Drugs. 1998 Sep;56(3):299-305. doi: 10.2165/00003495-199856030-00001. Erratum in: Drugs 1999 Jan;57(1):79. PMID: 9777308.
2. MEDICATION GUIDE Infliximab for injection, for intravenous use. (n.d.). Available at: https://www.janssenlabels.com/package-insert/product-patient-information/Infliximab-medication-guide.pdf.
3. Therapeutic drug monitoring countering the effect of anti-infliximab antibodies. Hooijberg, Femke et al. The Lancet Rheumatology, Volume 6, Issue 4, e193 - e194
4. Clinical consequences of infliximab immunogenicity and the effect of proactive therapeutic drug monitoring: exploratory analyses of the randomised, controlled NOR-DRUM trials. Brun, Marthe Kirkesæther et al. The Lancet Rheumatology, Volume 6, Issue 4, e226 - e236

TECHNICAL ASSISTANCE AND CUSTOMER SERVICE

For technical assistance or place an order, please contact Epitope Diagnostics, Inc. at (858) 693-7877 or fax to (858) 693-7678.

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Please visit our website at www.epitopediagnostics.com
to learn more about our products and services.

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GLOSSARY OF SYMBOLS (EN 980/ISO 15223)

RUO For Research Use Only	CE European Conformity	LOT Lot Number
REF Catalog Number	Read instructions before use	Number of Tests
Store at	Use by	Keep away from heat and direct sun light
Manufacturer	EC REP Authorized Representative in Europe	