cellsciences.com

Infliximab Level ELISA

Catalog No: M2920 **Lot No:** **Size:** 1 x 96 tests **Expiration Date:**

Specificity:	Infliximab - chimeric monoclonal antibody specific for TNFα (validated for Remicade® and the biosimilars Remsima® and Inflectra®)
Sensitivity:	0.08 µg/ml
Linear Range:	0.22 – 39.7 µg/ml
Sample Type:	Human plasma and serum
Cross-Reactivity:	No cross reactivity with TNF inhibitors adalimumab, etanercept and golimumab.
Notes:	**Cell Sciences' ELISA kits are not validated for IVD use**.

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INTRODUCTION

The Infliximab Level enzyme linked immunoassay (ELISA) is fast, reproducible, and gives specific quantitative determination of infliximab concentrations in human plasma and serum samples.

The therapeutic chimeric antibody, infliximab, affects tumor necrosis factor alpha (TNF) and is frequently administered to patients who suffer from rheumatic arthritis, intestinal disorders, dermatological diseases, and cancer. TNF plays an important role in inflammation; for example, it causes pain, swollen joints and stiffness in rheumatoid arthritis patients. Inhibition of TNF is therefore believed to relieve some of these symptoms and thus to improve quality of life of patients.

Plasma and serum levels of TNF inhibitors, such as infliximab, are highly variable between patients, and clearly correlate to the clinical symptoms in patients. In approximately 8%-43% of the patients treated with infliximab, antibodies are formed directed towards infliximab. This can partially hamper the function of the TNF inhibitor and can cause a reduction in its plasma concentration. This infliximab ELISA has been developed for fast, reproducible and specific quantification of infliximab concentrations in plasma and serum, for use in studying the effects of patient response to infliximab treatment.

Principle of the Test

The infliximab ELISA is a "sandwich-type" of enzyme immunoassay. TNF is captured by monoclonal antibodies coated to polystyrene microtiter wells. The infliximab present in the sample, the calibrator, or the controls, binds to the TNF on the microtiter plate. Non-bound material is then removed by washing. Subsequently, a horseradish peroxidase (HRP)-labeled monoclonal anti-infliximab antibody is added. This antibody binds to the infliximab/ TNF/anti-TNF complex present in the microtiter well. After removal of non-bound HRP conjugate by washing, substrate solution is added to the wells. A colored product is formed in proportion to the amount of infliximab present in the sample, calibrator, and controls. After the reaction has been terminated by the addition of a stop solution, absorbance is measured in a microtiter plate reader. From the absorbance of samples and those of the calibrator curve, the concentration of infliximab can be determined by interpolation with the calibrator curve.

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REAGENTS PROVIDED: RECONSTITUTION & STORAGE GUIDELINES

REAGENTS	1 x 96 wells	RECONSTITUTION
Microtiter strip-plate pre-coated with mouse-anti-TNF/ recombinant human TNF	1 plate of 12 x 8-well strips	Ready for use.
Calibrator 1 - 6	6 x 1 ml (black caps)	Ready for use.
Control 1 (therapeutic range)	1 x 1 ml (clear cap)	Ready for use.
Control 2 (sub-therapeutic range)	1 x 1 ml (clear cap)	Ready for use.
Human anti-infliximab HRP-conjugate	1 x 12.5 ml (brown bottle)	Ready for use.
Wash buffer stock solution	1 x 50 ml (white bottle)	Dilute 1:20 in distilled water.
HPE dilution buffer	1 x 50 ml (white bottle)	Ready for use.
TMB substrate solution	1 x 12.5 ml (brown bottle)	Ready for use.
Stop solution 0.18M H2SO4	1 x 13.5 ml (white bottle)	Ready for use.
Plate seals	10x	

STORAGE INSTRUCTIONS

- Store the kit reagents between 2 and 8°C. Immediately after use, remaining reagents should be returned to cold storage (2-8°C).
- Expiration date of the kit and reagents is stated on box front labels. DO NOT use reagents beyond expiration date. The expiration date of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use, the reagent is not contaminated during handling.
- Determine the number of strips required to test the desired number of samples, plus 8 wells needed for running calibrators and controls. Remove strips that will not be used from the microtiter plate-frame and store them in the plastic pouch containing the desiccant, at 2-8°C. Well strips are stable for up to 4 weeks after opening, if stored as indicated.

MATERIALS/ REAGENTS REQUIRED BUT NOT PROVIDED

- Distilled or deionized water.
- Calibrated pipettes (5 -1000µl).
- Multichannel pipette (30 – 300µl).
- Beakers, flasks, cylinders, and liquid containers necessary for preparation of reagents.
- Microtiter plate reader (for reading OD at 450nm).

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SAFETY AND PRECAUTIONS FOR USE

- Leaking or damaged vials cannot be used.
- Reagents (unopened or opened) cannot be used beyond the expiration date.
- Do not use samples with interference factors higher than indicated in the “Specifications” section of this protocol.
- All samples must be considered as potentially infectious. Handle all plasma and serum samples with care to prevent transmission of blood-borne infections.
- Care must be taken in the use and disposal of each container and its contents. Waste-disposal, after completion of the test, must be performed according to your laboratory regulations.
- None of the materials and reagents in the kit require a Safety Data Sheet (MSDS).

SPECIMEN COLLECTION, PROCESSING & STORAGE

- “Trough” samples must be used to measure the concentration of infliximab. Samples must be taken within 24 hours BEFORE the drug is injected to make sure that the indicated expected levels reflect the trough level of the patient.
- Only serum and plasma (Li-heparin and EDTA) can be used in the assay.
- Separate plasma or serum from the blood cells within 4 hours after collection and perform the analyses immediately. If testing of the samples is delayed, they can be stored at 2-8°C for 24 hours or frozen at ≤-20°C for 2 months. Aliquot samples to **avoid freeze-thaw cycles.**
- Prior to the assay, frozen samples must be thawed at room temperature. Do not use 37°C or 56°C water baths for thawing.
- Mix the samples just before preparing the dilutions.

ASSAY PREPARATION

Dilution of Samples

1. All samples should be diluted in duplicate using HPE dilution buffer.
2. A dilution of 1:1500 can be used in this assay to measure infliximab levels in patients.
3. If the concentration of the sample is too high to obtain an exact concentration, repeat the test with a sample dilution of 1:2000 to obtain a reliable result.
4. If the concentration of the sample is too low to obtain an exact concentration, repeat the test with a sample dilution of 1:200 to obtain a reliable result.

Dilution	Sample volume/ type	HPE volume
1:50	5µl of undiluted patient sample	245µl
1:200	50µl of 1:50 pre-diluted patient sample	150µl
1:1500	10µl of 1:50 pre-diluted patient sample	290µl
1:2000	5µl of 1:50 pre-diluted patient sample	195µl

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ASSAY PREPARATION (continued)

Preparation of Wash Buffer

Prepare a working-strength solution by adding 50ml of the Wash Buffer Stock Solution (this is the total volume of one bottle) to 950ml of distilled water.

The working-strength solution can be stored up to 2 months at 2-8°C.

Proposed Microtiter Plate Layout

Note: The calibration curve and controls must be included for each quantitative analysis run, they can be performed in one single row. The reagents provided give the user the possibility to use the microtiter plate in up to four runs. A proposed microtiter plate layout is given for use in one single run.

	1	2	3	4	5	6	7	8	9	10	11	12
A	CAL1	sample 1	sample 5	sample 9	sample 13	sample 17	Sample 21	sample 25	sample 29	sample 33	Sample 37	sample 41
B	CAL2	sample 1	sample 5	sample 9	sample 13	sample 17	sample 21	sample 25	sample 29	sample 33	sample 37	sample 41
C	CAL3	sample 2	sample 6	sample 10	sample 14	sample 18	sample 22	sample 26	sample 30	sample 34	sample 38	sample 42
D	CAL4	sample 2	sample 6	sample 10	sample 14	sample 18	sample 22	sample 26	sample 30	sample 34	sample 38	sample 42
E	CAL5	sample 3	sample 7	sample 11	sample 15	sample 19	sample 23	sample 27	sample 31	sample 35	sample 39	sample 43
F	CAL6	sample 3	sample 7	sample 11	sample 15	sample 19	sample 23	sample 27	sample 31	sample 35	sample 39	sample 43
G	CTRL 1	sample 4	sample 8	sample 12	sample 16	sample 20	sample 24	sample 28	sample 32	sample 36	sample 40	sample 44
H	CTRL 2	sample 4	sample 8	sample 12	sample 16	sample 20	sample 24	sample 28	sample 32	sample 36	sample 40	sample 44

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ASSAY PROTOCOL

- Allow all reagents to reach room temperature (18–25°C).
- The complete assay must be performed at room temperature (18-25°C) without shaking.
- Do not allow wells to stand uncovered or dry for extended periods between incubation steps.
- Carefully remove all air bubbles from the wells before incubation.
- To avoid cross-contamination, use disposable pipette tips for each transfer and use new plate seals for each incubation/fixation step in the ELISA experiment.
- Mix all reagents thoroughly but gently before use (without foaming).

Steps	Protocol Details
1.	Remove the microtiter plate with the required number of microtiter plate strips from the pouch. The unused strips can be stored in the plastic pouch with the desiccant.
2.	Prepare the wash buffer and the samples according to protocol.
3.	Add 100µl per well of calibrators, controls, or diluted patient samples according to the proposed microtiter plate layout (or your own layout). Close the vials of the calibrators and controls after use, to prevent evaporation.
4.	Cover the microtiter plate with adhesive seal and incubate for 1 hour.
5.	Aspirate supernatants from wells and fill each well with 250µl of diluted wash buffer. Aspirate the wash buffer. Repeat this step 4 times. After the final wash the wells must be dry!
6.	Add 100µl of the anti-infliximab HRP-conjugate to each well.
7.	Cover microtiter plate with adhesive seal and incubate for 1 hour.
8.	Aspirate supernatants from wells and fill each well with 250µl of diluted wash buffer and aspirate the wash buffer. Repeat this step 4 times. After the final wash the wells must be dry!
9.	Add 100µl of TMB substrate solution to each well.
10.	Incubate the microtiter plate in the dark and do not shake. Check the color formation every 5 minutes. When the blue color has developed in the positive wells and the blank is still colorless, the reaction must be stopped. The average incubation time is 10±1 minutes.
11.	Stop the reaction by adding 100µl of stop solution per well.
12.	Measure the microtiter plate in an ELISA reader at A450nm. Read the plate within 30 minutes after the stop solution is added.

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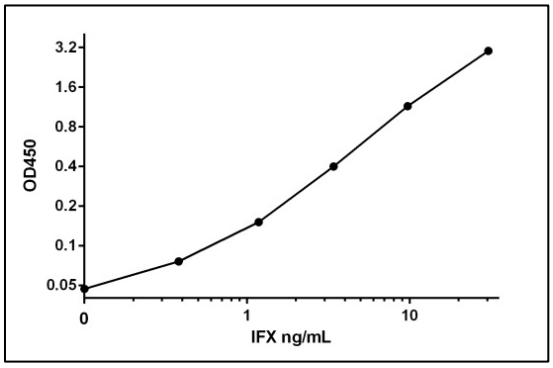
DATA ANALYSIS

An in-house or on-line available software method for calculation of concentrations may be used. A general method for calculation by hand is shown here:

1. Record the absorbance at 450nm for each well containing calibrator.
2. Plot the absorbance on the Y-axis on a linear scale and plot the infliximab concentration of the calibrator sample on the X-axis on a log scale and draw the best fitting curve.
3. Record the absorbance at 450nm for each well containing a specific sample.
4. Locate the net average absorbance value found for each sample on the vertical axis and follow a horizontal line intersecting the calibrator curve.
5. Draw a vertical line from the intersection of the calibrator curve towards the X-axis.
6. At the intersection with the X-axis, read the infliximab concentration from the horizontal axis.
7. Multiply the obtained infliximab concentration with the dilution factor of the sample, this is the actual concentration of infliximab in the sample. **NOTE**: For control 1 and control 2, use a dilution factor of 1:1500.
8. Calculate the average of the duplicate values when the sample is performed in duplicate.

Example of standard curve after 10 minutes of color formation:



Typical therapeutic levels for patients treated with infliximab*	
Healthy donors and patients not treated with infliximab	negative
Sub-therapeutic levels of infliximab	<3.0 µg/ml
Normal therapeutic levels of infliximab	3.0 – 7.0 µg/ml
Elevated levels of infliximab	>7.0 µg/ml

*Only indications for therapeutic values are given. Each laboratory must define its own cut-off values.

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TYPICAL SPECIFICATIONS

Recovery: 94% at 2 µg/ml (1:1500)

Limit of quantification:	lower limit:	0.08 µg/ml (1:200)
	upper limit (antigen excess):	no antigen access observed (47 µg/ml at 1:2000)

Precision	Total Precision	Between-run Precision
0.30 µg/ml (1:200)	11.0%	9.5%
2.14 µg/ml (1:1500)	8.8%	5.8%
17.3 µg/ml (1:2000)	7.4%	6.5%

Linear range: 0.22 – 39.7 µg/ml

Cross-reactivity: No cross reactivity with TNF inhibitors adalimumab, etanercept and golimumab.

Interference factors:	interference <20% with:	hemoglobin	5 and 40 mg/ml
		bilirubin conjugated	0.02 and 0.5 mg/ml
		bilirubin unconjugated	0.1 and 1.5 mg/ml
		triglycerides	15 and 50 mg/ml
		human serum albumin	60 and 80 mg/ml
		rheumatoid factor (RA)	1600 U/ml

Method comparison	assay: 70 patient samples were compared to the in-house validated method
	analysis: Passing and Bablock: y=0.91x+0.04, Spearman correlation: 0.99

Limitations

- The kit is designed for professional use. The user must be trained and familiar with ELISA test procedures.
- For optimal performance of ELISA make sure that all pipets and systems are checked and under full maintenance service according to described procedures of the manufacturers.
- This ELISA kit is only validated for manual use, as described in this document. If using the kit on an automated ELISA machine, the test must be validated by the user. Any claims in this document are invalid.
- Do not use the reagents after the expiration date.
- When the controls are not within the indicated range, the results are not valid and the test must be repeated.
- As the controls are pre-diluted, they cannot be used to check sample and reagent preparation by the user.
- Samples that have an OD450nm outside the calibrator curve are not valid and cannot be used for calculations; DO NOT extrapolate results. These samples must be measured in lower or higher dilutions.
- False positive or negative results can be obtained when samples are used with interference factors higher than indicated in the specifications.
- Only reagents supplied with the kit must be used. Do not use reagents from different batches or from different kit lots, as these are not interchangeable. Reagents or remnants of reagents (e.g. dead volume) cannot be mixed with contents of freshly opened vials.
- Caps and vials are not interchangeable, caps must be replaced on the corresponding vials.
- Sodium azide cannot be added to the reagents, this affects the performance of the test.
- Do not use aluminum foil during the incubation steps.

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