1. Intended Use
SHIKARI® Infliximab ELISA has been especially developed for the quantitative analysis of free-infliximab in serum and plasma samples. SHIKARI® Infliximab ELISA is compiled with Remicade®.

2. General Information
Infliximab is in a tumour necrosis factor (TNF) blocker and a chimeric monoclonal IgG1 antibody composed of human constant (75%) and murine variable (25%) N-glycosylated polypeptides. Infliximab is produced by a recombinant cell line cultivated by submerged perfusion. TNFα is a key proinflammatory cytokine involved in chronic inflammatory diseases. Its hyperactivity and enhanced signaling pathways can be detected in inflammatory diseases which lead to further proinflammatory reactions. By locally to test the suitable safety level of a biologic medicinal product in the form of a ELISA test kit. The indication for the use of a ELISA test kit is the determination of the presence of antibodies against the drug infliximab in a patient's sera. The test can be used in accordance to the national biohazard safety guidelines or regulations. Follow good laboratory practice (GMP). Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired kits. All reagents of this kit containing human serum or plasma (standards etc.) have been tested and were found negative for HIV I/II, HBsAg and Anti-HCV. However, a presence of these or other infectious agents cannot be conclusively excluded and therefore reagents should be treated as potential biohazards in use and for disposal. The user of the kit is responsible for the determination of the presence of biohazardous agents in the kit reagents. See “Materials supplied”, MSDS and labels for details.

3. Test Principle
Solid phase enzyme linked immunosorbent assay (ELISA) based on the sandwich principle. Standards and samples (penta or plasma) are incubated in the microtiter plate coated with the reagent for infliximab. After incubation, the wells are washed. Then, horse radish peroxidase (HRP) conjugated antibody is added and bound to the infliximab captured by the reagents on the surface of the wells. Following incubation wells are washed and the bound enzymatic activity is detected by addition of tetramethylbenzidine (TMB) chromogen substrate. Finally, the reaction is terminated with an acidic stop solution. The colour development is proportional to the amount of infliximab in the sample or standard. Results of samples can be determined directly using the standard curve.

4. Warnings and Precautions
- For professional use only.
- In case of serious damage the kit package please contact Matrix Biotek® or your supplier in written form. Label one week after receiving the kit. Do not use damaged components in tests but keep safe for completed related issues.
- Obey lot number and expiry date. Do not mix reagents of different lots.
- Before starting the test, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood. For further information (clinical background, test performance, automation protocols, alternative applications, literature, etc.) please refer to the local distributor.
- Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.
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5. Storage and Stability
The kit is shipped at ambient temperature (10-30°C) and should be stored at 2-8°C in long term storage. Keep away from heat in direct sunlight. The shelf life of microtiter plate is stable up to the expiry date on the packaging, but tightly closed bags when stored at 2-8°C 2 years.

6. Spectrum (Collection and Storage)
Serum (Phlebotomy): Twenty micro litres of serum is necessary and collected in a glass tube containing 0.1 volumes glycerol. The samples should be frozen and stored for up to 3 months at -70°C.

7. Materials Supplied

<table>
<thead>
<tr>
<th>Product Code</th>
<th>Description</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>INF-FD-REMI</td>
<td>Internal (Reagent)</td>
<td>Free drug</td>
</tr>
<tr>
<td>INF-QS-REMI</td>
<td>Antibody screening</td>
<td>Qualitative</td>
</tr>
<tr>
<td>INF-QN-REMI</td>
<td>Wash Buffer</td>
<td>Neutralised</td>
</tr>
<tr>
<td>INF-QT-REMI</td>
<td>Standards A</td>
<td>Free/Total semi quantitative</td>
</tr>
<tr>
<td>INF-QT-REMI</td>
<td>Standards B</td>
<td>Free/Total semi quantitative</td>
</tr>
<tr>
<td>INF-QT-REMI</td>
<td>Standar E</td>
<td>Free/Total semi quantitative</td>
</tr>
</tbody>
</table>

8. Materials Required but Not Supplied
- Microplates and tips
- Collected measures
- Tubs for sample dilution
- Wash bottle, automated or semi-automated microtiter plate washing system
- Microplate reader capable of measuring optical density with a photometer at OD 450µm with reference wavelength 630 nm (450/630 nm)
- Distilled or deionised water, paper towels, pipette tips and timer

9. Procedure Notes
- Any improper handling of samples or modification of the test procedure may influence the results. The included pipetting volumes, incubation times, temperature and pre-treatment steps must be performed strictly according to the instructions. Use calibrated pipettes and devices only.
- Once the test has been started, all steps of the procedure must be completed without interruption. Make sure that required reagents, materials and devices are prepared ready at the appropriate time. Allow all reagents and samples to reach room temperature (10-25°C) and gently swirl each well of liquid sample and reagent before use. Mix reagents without rocking.
- Avoid contamination of reagents, pipettes, and vials. Use new disposable plastic pipette tips for each reagent, standard of sample. Do not re-use tips. Always keep caps on vials. Do not reuse vials or reagents.
- Use a pipetting scheme to verify an appropriate plate layout.
- The number of samples and standards should be balanced in the same order and time sequences. It is recommended to use an eight-channel micropipette for planting of solutions in all wells.
- Microplate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a microplate washer or an automatic microplate washing system. Do not allow the wells to dry between incubations. Do not soak controlled plates during any step after the addition of reagents. Always wash with reagent buffer after mixing. Check that all wells are filled perfectly with wash buffer, and that there are no residues in the wells.
- Humidity affects the coated wells/tubes. Do not open the kit until it reaches room temperature. Unused vials/tubes should be returned immediately to the resealig pouch including the desiccant.
10. Pre-test Setup Instructions

- Preparation of components

<table>
<thead>
<tr>
<th>Component</th>
<th>Wash buffer (must be prepared before starting assay procedure)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dilute</td>
<td>10 mL (4 g/L)</td>
</tr>
<tr>
<td>With</td>
<td>Serum/Plasma</td>
</tr>
<tr>
<td>Diluent</td>
<td>0.1 M sodium phosphate (pH 7.5)</td>
</tr>
</tbody>
</table>

Dilution Ratio 1:100

Remarks: Warm up to 37°C to dissolve crystals. Mix vigorously.

Storage: 2-8°C

Stability: 2 weeks

- Dilution of samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Serum/Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diluent</td>
<td>Assay buffer</td>
</tr>
<tr>
<td>Dilution Ratio</td>
<td>1:100</td>
</tr>
</tbody>
</table>

11. Test Procedure

<table>
<thead>
<tr>
<th>Dilute 10 µL into each of the wells to be used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pipette 100 µL “Assay Buffer” into each well</td>
</tr>
<tr>
<td>Pipette 10 µL of each “Standard”, “Low level control”, “High level control” and diluted samples into the respective wells of microtiter plate</td>
</tr>
</tbody>
</table>

Wells:
- A1: Standard A
- B1: Standard B
- C1: Standard C
- D1: Standard D
- E1: Standard E
- F1: Low level control
- G1: High level control

1. Incubate 30 minutes at room temperature (18-25°C)

2. Cover the plate with adhesive foil

3. Briefly mix contents by gently shaking the plate

4. Remove excess solution by tapping the inverted plate on a paper towel

5. Pipette 100 µL “Corrigate” into each well

6. Incubate 45 minutes at room temperature (18-25°C)

7. Cover the plate with adhesive foil

8. Remove excess solution by tapping the inverted plate on a paper towel

9. Pipette 100 µL “Substrate” into each well

10. Incubate 10 minutes without adhesive foil at room temperature (18-25°C) in the dark

11. Stop the substrate reaction by adding 100 µL “Stop Solution” into each well

12. Measure optical density at a photometer at OD 450 nm with reference wavelength 650 nm (645 nm) within 20 minutes after pipetting the “Stop Solution”

13. Calculation and Interpretation of Results

- Create a standard curve by using the standards: OD 400/650 nm for each standard on the vertical Y-axis and versus the corresponding drug concentration on the horizontal (X-axis) axis.

- The concentration of the sample can be read directly from the standard curve. Using the absorbance value for each sample, determine the corresponding concentration of drug from the standard curve. Plot the absorbance value on the Y-axis and extend a horizontal line to the curve. At the point of intersection, extend a vertical line to the X-axis and read the drug concentration of the unknown sample.

- If computer data is going to be used, we recommend primarily “Four Parameter Logistic (LR4)” or secondarily the “Three-point-point calculation”.

- To obtain the exact values of the samples, the concentration determined from the standard curve must be multiplied by the dilution factor (df). Any sample reading greater than the highest standard should be further diluted appropriately with assay buffer and retested. Therefore, if the pre-diluted samples have been further diluted, the concentration determined from the standard curve must be multiplied by the further dilution factor.

- e.g.: If the pre-diluted sample further diluted in a ratio of 1:15 then results should be multiplied by 100.

- For low and high level controls values, refer to “Quality Control Certificate” provided by each kit.

14. Analytical Performance

- Calibration curve (Linearity, Dilutional linearity): Y > 0.95

- Precision: Intra assay CVs < 15.0%

- Inter assay CVs < 15.0%

- Recovery <100±30%

- Functional sensitivity (Limit of quantification - LOQ): 0.005 µg/mL (5 ng/mL)

15. Automation

SHIKAR® Infliximab ELISA is also suitable to run on automated ELISA processors.