# *Manual*
# TL1A soluble (human), detection set

**[TNFSF 15, Soluble (human) Detection Set]**

***For Research Use Only***

**APO-54N-024**

**Version 4 (29-Apr-13)**

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## TRADEMARKS AND PATENTS

Several Enzo Life Sciences products and product applications are covered by US and foreign patents and patents pending.

## 1. INTENDED USE

The **TL1A soluble (human), detection set** is to be used for the *in vitro* quantitative determination of soluble human TL1A in biological fluids. This Detection Set is for research use only.

## 2. INTRODUCTION

TL1A, a ligand belonging to the tumor necrosis factor (TNF) family (also called TNFSF15), is expressed predominantly by endothelial cells, but is also found on lymphocytes, plasma cells and monocytes. TL1A is upregulated by the proinflammatory cytokines TNF and IL-1. On activated T cells, TL1A functions specifically via its surface-bound receptor DR3, (a member of the death-domain containing TNF receptor family) to promote cell survival and secretion of proinflammatory cytokines (1). The decoy receptor 3 (DcR3), a soluble protein of the tumor necrosis factor receptor (TNFR) superfamily blocks the action of TL1A (2). TL1A, like TNF, can be released to circulate as a homotrimeric soluble form (3). Activation of DR3 by TL1A induces the formation of a signaling complex containing TRADD, TRAF2, and RIP and activates the NF-kappaB and the ERK, JNK, and p38 mitogen-activated protein kinase pathways (4). The TL1A/DR3 pathway may be involved in atherosclerosis via the induction of pro-inflammatory cytokines/chemokines (5) and seems to play an important role in Th1-mediated intestinal diseases, such as Crohn's disease (6).

1. Migone, T. S. et al. TL1A is a TNF-like ligand for DR3 and TR6/DcR3 and functions as a T cell costimulator. Immunity 16, 479-92 (2002).
2. Hsu, M. J. et al. Enhanced adhesion of monocytes via reverse signaling triggered by decoy receptor 3. Exp Cell Res 292, 241-51 (2004).
3. Kim, S. & Zhang, L. Identification of naturally secreted soluble form of TL1A, a TNF-like cytokine. J Immunol Methods 298, 1-8 (2005).
4. Wen, L., Zhuang, L., Luo, X. & Wei, P. TL1A-induced NF-kappaB activation and c-IAP2 production prevent DR3-mediated apoptosis in TF-1 cells. J Biol Chem 278, 39251-8 (2003).
5. Kang, Y. J. et al. Involvement of TL1A and DR3 in induction of pro-inflammatory cytokines and matrix metalloproteinase-9 in atherogenesis. Cytokine 29, 229-35 (2005).
6. Bamias, G. et al. Expression, localization, and functional activity of TL1A, a novel Th1-polarizing cytokine in inflammatory bowel disease. J Immunol 171, 4868-74 (2003).

## 3. PRINCIPLE OF PROCEDURE

This assay is a sandwich Enzyme-Linked ImmunoSorbent Assay (ELISA) developed for the direct measurement of human TL1A (hTL1A) in biological fluids. A monoclonal antibody specific for hTL1A (COAT) is coated onto the wells of the microtiter plate. Samples and standards of hTL1A are pipetted into the wells for binding to the coated antibody. After extensive washing to remove unbound compounds, hTL1A is recognized by the addition of a biotinylated monoclonal antibody specific for hTL1A (DET). After removal of excess biotinylated antibody, streptavidin-peroxidase is added. Following a final washing, peroxidase activity is quantified using the substrate 3,3',5,5'-tetramethylbenzidine (TMB). The intensity of the color reaction is measured at 450 nm after acidification and is directly proportional to the concentration of hTL1A in the samples.

## 4. MATERIALS PROVIDED

- 1 vial Standard (*lyophilized*) (2 µg) (STD) Product No. 80-2118
- 1 vial Coating Antibody (120µl) (COAT) Product No. 80-2337
- 1 vial Detection Antibody (60µl) (DET) Product No. 80-2338

## 5. MATERIALS REQUIRED

- PBS*: 100 mM $PO_4$, pH 7.1, 75 mM NaCl
- Wash Buffer*: 0.1% Tween® 20 in PBS
- ELISA Buffer*: 1% BSA and 0.1% Tween® 20 in PBS
- Blocking Buffer*: 1% BSA in PBS
- 1N Hydrochloric acid (HCl), such as Product No. ADI-80-1804 contained in the ImmunoSet™ Buffer Pack
- Tetramethylbenzidine substrate (TMB), such as Product No. ADI-80-1805 contained in the ImmunoSet™ Buffer Pack
- HRP-Labeled Streptavidin (SA-HRP), such as Product No. ADI-80-1896
- HPLC grade water

*These buffer formulations can be easily prepared using solutions of PBS concentrate (ADI-80-1927), 10% BSA (ADI-80-1928), and Tween® 20 (ADI-80-1929) contained in the ImmunoSet™ Buffer Pack, Product No. ADI-950-003

## 6. PRODUCT SPECIFICATION

**Number of Assays:** This Detection Set contains sufficient materials to run ELISAs on 5 x 96-well plates.

**Specificity:** The monoclonal antibodies used in this detection Set are specific for measurement of natural and recombinant human TL1A. They do not cross-react with mouse TL1A.

**Sensitivity:** 20 pg/ml (range 0 to 2.5 ng/ml)

**Stability:** Components as provided are stable for at least 3 months at 4°C. For long term storage, keep the antibodies and standard at -20°C. Avoid freeze/thaw cycles

# 7. GENERAL ELISA PROTOCOL

## PREPARATION OF REAGENTS

1. Dilute the desired amount of Coating Antibody (COAT) (1 mg/ml) to 2 µg/ml in PBS.
2. Dilute the desired amount of Detection Antibody (DET) (1 mg/ml) to 1 µg/ml in ELISA Buffer.
3. Dilute the desired amount of HRP-Labeled Streptavidin (follow manufacturer's recommendations for use). If using SA-HRP Product No. ADI-80-1896, reconstitute the vial with 0.25 ml of $dH_2O$, then dilute 1:1000 in ELISA buffer.
4. Reconstitute the Standard Protein (STD) with 100 µl ELISA buffer to obtain a concentration of 0.02 mg/ml. **Store the reconstituted standard at or below -20°C!** A seven point standard curve using 2-fold serial dilutions in ELISA buffer is recommended. Suggested standard points are 2.5ng/ml, 1.25ng/ml, 0.625ng/ml, 0.312ng/ml, 0.156ng/ml, 0.078ng/ml, and 0.039ng/ml.
5. If measuring serum or cell culture supernatant, dilute samples in ELISA buffer.
6. To reduce interference from rheumatoid factor (RF) in the serum, Ig can be cleared by treating serum dilutions with 0.05 volume of protein G-Sepharose (such as GE Healthcare cat. 17-0618-01) twice for one hour at 4°C before pelleting the beads and collecting supernatants (precleared sera)[1].

## PLATE PREPARATION

1. Coat the wells by adding 100µl/well of diluted (2 µg/ml) Coating Antibody (COAT) to a 96-well ELISA microplate. We recommend our ImmunoSet™ Plate Pack, Product No. ADI-80-1930, which contains five 96-well clear flat-bottom microtiter plates with sealers. Cover the plate with plastic film and leave overnight at 4°C.
2. Aspirate the coated wells and add 400 µl of Wash Buffer using a multichannel pipette or autowasher. Repeat the process for a total of four washes. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or by inverting the plate and blotting it against clean paper towels.
3. Block plates by adding 200 µl of Blocking Buffer at RT for 2 to 4 hours.
4. Repeat the aspiration/wash as in step 2 for a total of four washes.

## ASSAY PROCEDURE

1. Add a total of 100 µl/well diluted serum, diluted culture supernatant or Standard Protein serial dilutions in ELISA Buffer to the plate.
2. Cover the plate with plastic film and incubate for one hour at RT.
3. Repeat the aspiration/wash as in step 2 of “Plate Preparation” for a total of four washes.
4. Add 100 µl/well of the diluted (1 µg/ml) Detection Antibody (DET).
5. Cover the plate with plastic film and incubate for one hour at RT.
6. Repeat the aspiration/wash as in step 2 of “Plate Preparation” for a total of four washes.
7. Add 100 µl to each well of the diluted HRP-Labeled Streptavidin.
8. Cover the plate with plastic film and incubate for 30 min at RT.
9. Aspirate the coated wells and add 400 µl of Wash Buffer using a multichannel pipette or autowasher. Repeat the process for a total of four washes. After the last wash, complete removal of liquid is essential for good performance.
10. Substrate development is conducted using tetramethylbenzidine substrate (follow manufacturer’s recommendations for use). If using TMB Product No. ADI-80-1805, an incubation of 15 – 30 minutes at RT is recommended.
11. Stop the reaction by adding 100µl of 1N HCl. Tap the plate gently to ensure thorough mixing.
12. Measure the OD at 450 nm in an ELISA reader.

## 8. TECHNICAL HINTS AND LIMITATIONS

- Once reagents have been added to the plate, DO NOT let the plate dry at any time during the assay.
- Be sure no sodium azide is present in this assay, as this inhibits HRP enzyme activity.
- It is recommended that all standards and samples be assayed in duplicate.
- Avoid microbial contamination of reagents and equipment. Automated plate washers can become contaminated thereby causing assay variability. Buffers containing a large quantity of protein should be made under sterile conditions and stored at 2-8°C or be prepared fresh daily.
- Vigorous plate washing is essential.
- Avoid exposing reagents to excessive heat or light during storage and incubation.
- Wear gloves while performing the assay to avoid contact with samples and reagents. Please follow proper disposal procedure.

## 9. CALCULATION OF RESULTS

- Average the duplicate readings for each standard, control and sample and subtract the average blank value.
- Generate the standard curve by plotting the average absorbance obtained for each standard concentration on the vertical (Y) axis vs. the corresponding hTL1A concentration (ng/ml) on the horizontal axis (see **TYPICAL DATA**).
- Calculate results using graph paper or curve-fitting statistical software. We recommend that the data be handled by an immunoassay software package utilizing a four parameter logistic curve fitting program (such as AssayBlaster™, Product No. ADI-28-0002). Such software is often supplied by plate reader manufacturers. The amount of hTL1A in each sample is determined by interpolating for the absorbance value (Y axis) using the standard curve.
- If the test sample was diluted, multiply the interpolated value by the dilution factor to calculate ng/ml of human TL1A in the sample.

## 10. TYPICAL DATA

The following data are obtained using the different concentrations of standard as described in this protocol:

| Standard hTL1A (ng/ml) | Optical Density (mean) |
|---|---|
| 0 | 0.093 |
| 0.039 | 0.124 |
| 0.078 | 0.127 |
| 0.156 | 0.167 |
| 0.312 | 0.250 |
| 0.625 | 0.400 |
| 1.25 | 0.714 |
| 2.5 | 1.495 |

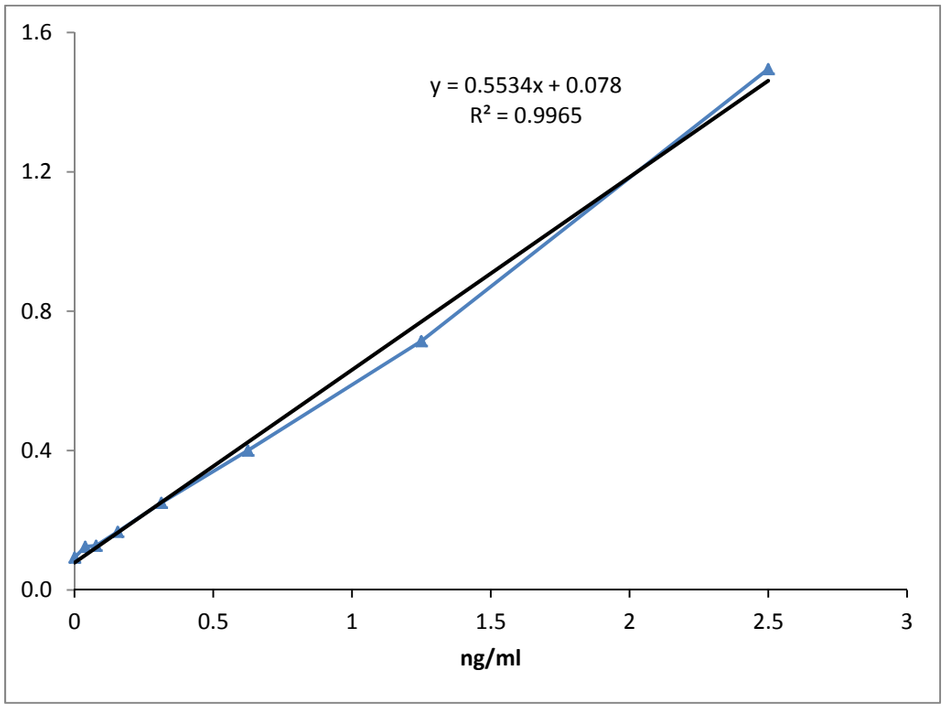


Figure: Standard curve

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