



**Highest Standards for  
Research Kits™**

***Manual  
ILEI (human) Detection Set  
(for ELISA Application)***

**[Interleukin-like EMT Inducer (human) Detection Set ;  
FAM3C (human) Detection Set]**

***For Research Use Only***

**APO-54N-032**

**Version 1 (30-Jan-08)**



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## 1. INTENDED USE

The ILEI (human) Detection Set is to be used for the *in vitro* quantitative determination of human ILEI in cell supernatant, plasma and serum. This Detection Set is for research use only.

## 2. INTRODUCTION

ILEI (Interleukin-like EMT-inducer, also called FAM3C) is a new secreted cytokine-like protein of 25 kDa that has structural homology to four-helix-bundle cytokines such as erythropoietin, growth hormone or interleukin members [1]. There are four genes in this new family : FAM3A, FAM3B, FAM3C (ILEI) and FAM3D [1]. ILEI possess a novel GG domain (because of two well-conserved glycine residues) present in eukaryotic FAM3 family, POMGnT1 (protein O-linked mannosyltransferase), TEM2 proteins as well as phage gp35 proteins [2]. While human FAM3B and D are expressed in a few tissues (such as pancreas and placenta), human FAM3A and FAM3C / ILEI are ubiquitously expressed. Recently, ILEI has been identified as a key player in epithelial-to-mesenchymal transition (EMT), in carcinoma progression as well as in metastasis [3]. ILEI acts downstream of TGF $\beta$  receptor signaling and is regulated at the level of translation during EMT [3]. ILEI expression is increased in many carcinomas and could serve as an independent prognostic marker in breast cancer [3]. ILEI is also secreted by pancreatic cancer cells [4, 5].

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2. Pilipenko, V.V., Reece, A., Choo, D.I., and Greinwald, J.H., Jr. (2004). Genomic organization and expression analysis of the murine Fam3c gene. *Gene* *335*, 159-168.
3. Waerner, T., Alacakaptan, M., Tamir, I., Oberauer, R., Gal, A., Brabletz, T., Schreiber, M., Jechlinger, M., and Beug, H. (2006). ILEI: a cytokine essential for EMT, tumor formation, and late events in metastasis in epithelial cells. *Cancer cell* *10*, 227-239.
4. Gronborg, M., Kristiansen, T.Z., Iwahori, A., Chang, R., Reddy, R., Sato, N., Molina, H., Jensen, O.N., Hruban, R.H., Goggins, M.G., et al. (2006). Biomarker discovery from pancreatic cancer secretome using a differential proteomic approach. *Mol Cell Proteomics* *5*, 157-171.
5. Mauri, P., Scarpa, A., Nascimbeni, A.C., Benazzi, L., Parmagnani, E., Mafficini, A., Della Peruta, M., Bassi, C., Miyazaki, K., and Sorio, C. (2005). Identification of proteins released by pancreatic cancer cells by multidimensional protein identification technology: a strategy for identification of novel cancer markers. *Faseb J* *19*, 1125-1127.

### 3. PRINCIPLE OF PROCEDURE

This assay is a sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA) developed for the direct measurement of human ILEI (hILEI) in biological fluids. A monoclonal antibody specific for hILEI (COAT) is coated onto the wells of the microtiter plate. Samples and standards of ILEI are pipetted into the wells for binding to the coated antibody. After extensive washing to remove unbound compounds, hILEI is recognized by the addition of a biotinylated monoclonal antibody specific for hILEI (DET). After removal of excess biotinylated antibody, streptavidine-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 hILEI in the samples.

### 4. MATERIALS PROVIDED

- 1 vial Standard (*lyophilized*) (1  $\mu$ g) (STD) APO-54N-032 /STD
- 1 vial Coating Antibody (260 $\mu$ l) (COAT) APO-54N-032 /COAT
- 1 vial Detection Antibody (120 $\mu$ l) (DET) APO-54N-032 /DET

### 5. MATERIALS REQUIRED

- PBS: 137mM NaCl, 2.7mM KCl, 8.1mM Na<sub>2</sub>HPO<sub>4</sub>, 1.5mM KH<sub>2</sub>PO<sub>4</sub>, pH 7.2, 0.2 $\mu$ M filtered
- Wash Buffer: 0.1% Tween<sup>®</sup> 20 in PBS
- Diluent Buffer: 2% BSA in PBS, 0.2 $\mu$ M filtered.
- ELISA Buffer: 0.2% BSA and 0.05% Tween<sup>®</sup> 20 in PBS
- 2M sulphuric acid (H<sub>2</sub>SO<sub>4</sub>)
- Tetramethylbenzidine substrate kit (SeraCare Life Sciences, code 91000-01K)
- Streptavidin-peroxidase (Jackson immunoresearch, N° 016-030-084)
- HPLC grade water
- Nunc MaxiSorp<sup>TM</sup> flat-bottom 96 well plate

### 6. PRODUCT SPECIFICATION

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

**Specificity:** The antibodies used in this detection Set are specific for measurement of natural and recombinant human ILEI.

**Sensitivity:** 0.2ng/ml (range 0 to 20 ng /ml)

**Stability:** Stable at least 6 months after receipt when stored at +4°C.  
For long term storage, keep the standard (STD) at -20°C

## 7. GENERAL ELISA PROTOCOL

### PREPARATION OF REAGENTS

1. Dilute the desired amount of Coating Antibody (COAT) (1 mg/ml) to 5 µg/ml in PBS without carrier protein.
2. Dilute the desired amount of Detection Antibody (DET) (1 mg/ml) to 2 µg/ml in ELISA buffer.
3. Reconstitute the Standard Protein (STD) with 100 µl PBS to obtain a concentration of 10 µg/ml. **After reconstitution, prepare aliquots and store the reconstituted standard at -20°C! Avoid freeze/thaw cycles !** A standard curve using 2-fold serial dilutions in ELISA buffer is recommended. Suggested standard points are 20 ng/ml, 10 ng/ml, 5 ng/ml, 2.5 ng/ml, 1.25 ng/ml, 0.625 ng/ml, 0.312 ng/ml and 0 ng/ml.
4. If measuring plasma, serum or cell culture supernatant, dilute samples in ELISA buffer.
5. To reduce interference from rheumatoid factor in the serum, Ig can be cleared by treating serum dilutions with 0.05 volume of protein G-Sepharose twice for one hour at 4°C before pelleting the beads and collecting supernatants.

### PLATE PREPARATION

1. Coat the wells by adding 100 µl/well of diluted (5 µg/ml) Coating Antibody (COAT) to a 96-well ELISA microplate (Nunc MaxiSorp™ flat-bottom 96 well plate is suggested). Cover the plate with plastic film and leave overnight at 4°C.
2. Aspirate the coated wells and add 300 µl of *Wash Buffer* using a multichannel pipette or autowasher. Repeat the process for a total of five 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 300 µl of Diluent Buffer at RT for 2 hours.
4. Repeat the aspiration/wash as in step 2 for a total of five washes.

## ASSAY PROCEDURE

1. Add a total of 100µl/well diluted plasma, serum, culture supernatant or Standard Protein serial dilutions in ELISA Buffer to the plate.
2. Cover the plate with plastic film and incubate for 1 hour at RT°C.
3. Aspirate the coated wells and add 300µl of *Wash Buffer* using a multichannel pipette or autowasher. Repeat the process for a total of five 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.
4. Add 100µl/well of the diluted (2µg/ml) Detection Antibody (DET).
5. Cover the plate with plastic film and incubate for 1 hour at RT°C.
6. Repeat the aspiration/wash as in step 3 for a total of five washes.
7. Add 100 µl to each well of the diluted HRP Labelled Streptavidin (from Jackson immunoresearch, dilute 1/10'000).
8. Cover the plate with plastic film and incubate for 30 min at RT°C.
9. Aspirate the coated wells and add 300 µl of Wash Buffer (Wash Buffer 1X) using a multichannel pipette or autowasher. Repeat the process for a total of five washes. After the last wash, complete removal of liquid is essential for good performance.
10. Substrate development is conducted by addition of 100µl to each well of ready-to-use tetramethylbenzidine (TMB) (SeraCare Life Sciences) for 20-30 min.
11. Stop the reaction by adding 50µl of 2M H<sub>2</sub>SO<sub>4</sub>. Tap the plate gently to ensure thorough mixing.
12. Measure the OD at 450nm in an ELISA reader.
13. Measure absorbance at 550nm and subtract these values from those obtained at 450nm to correct for optical imperfections in the microplate. If absorbance at 550nm is not possible, measure the absorbance at 450nm only.

**Note:** When the 550 nm measurement is omitted, absorbance values will be higher.

## 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 hILEI concentration (ng/ml) on the horizontal axis (see **TYPICAL DATA**). Calculate results using graph paper or curve-fitting statistical software. The amount of hILEI 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 ILEI in the sample.

### 10. TYPICAL DATA

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

Standard hILEI (ng/ml)	Optical Density (mean)
0	0.116
0.312	0.125
0.625	0.157
1.25	0.201
2.5	0.292
5	0.481
10	0.852
20	1.524

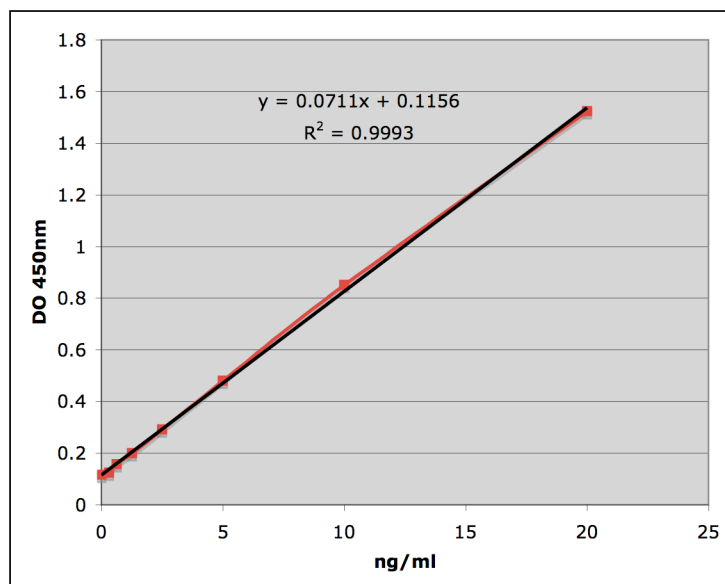


Figure: Standard curve



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