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MANUAL

IL-37 (human) ELISA Kit

For research use only. Not for diagnostic use.

Version 3 (05-May-2015)

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Product Specific References

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1. Intended Use

The IL-37 (human) ELISA Kit is to be used for the *in vitro* quantitative determination of human IL-37 in serum and plasma. This ELISA Kit is for research use only.

2. Introduction

The cytokine interleukin-1 elicits a wide array of biologic activities that initiate and promote the host response to injury or infection by activating a set of transcription factors, including NF κ B and AP1, which in turn induce production of effectors of the inflammatory response. By computational cloning a cDNA encoding IL-1F7 was isolated to some homology to IL-1 originally called FIL1-zeta. The deduced 192-amino acid protein contains a 15- to 30-amino acid prodomain. Although there are multiple splicing variants, IL-1F7b isoform is a 30 kDa protein and shares critical amino acid residues with IL-18 (1-2). After rigorous activity tests IL-1F7 was officially designated IL-37 (3). IL-37 mRNA has been detected in various hematopoietic organs as well as in other tissues (1). It has been shown that IL-37 is strongly expressed intracellularly in human monocytes whose expression can be further upregulated by LPS (4). IL-37 was shown to bind to the IL-18R without eliciting signal transduction (5). An immunologic capability associated with IL-37 was shown that intratumoral expression of IL-37 by adenovirus induces Th1-dependent anti-tumor immunity (6). Although IL-37 does not contain a canonical signal peptide at the NH₂-terminus, multiple protein species of IL-37 were detected in the culture supernatants with an overexpression of IL-37 (6). One interesting feature IL-37 exhibits is that upon LPS stimulation the NH₂-terminal prodomain encompassing 1st-45th amino acid residues is cleaved by caspase-1 and the resulting mature IL-37 actively translocates into the nucleus, suppressing the induction of TNF- α , IL-6, and MIP-2. Interaction of Smad3 with the translocated IL-37 has been an acting mechanism for the IL-37-mediated anti-inflammation (3), suggesting that IL-37 is a potent inhibitor of innate immunity. Measurement of intracellular IL-37 upon danger signals or proinflammatory cues would provide a novel insight into anti-inflammation.

3. General References

- (1) Four new members expand the interleukin-1 superfamily: D.E. Smith, et al.; J. Biol. Chem. **275**, 1169 (2000)
- (2) A Complex of the IL-1 homologue IL-1F7b and IL-18-binding protein reduces IL-18 activity: P. Bufler, et al.; PNAS **99**, 13723 (2002)
- (3) IL-37 is a fundamental inhibitor of innate immunity: M.F. Nold, et al.; Nat. Immunol. **11**, 1014 (2010)
- (4) Interleukin-1 homologues IL-1F7b and IL-18 contain functional mRNA instability elements within the coding region responsive to lipopolysaccharide: P. Bufler, et al.; Biochem. J. **381**, 503 (2004)
- (5) Interleukin-1F7B (IL-1H4/IL-1F7) is processed by caspase-1 and mature IL-1F7B binds to the IL-18 receptor but does not induce IFN-gamma production: S. Kumar, et al.; Cytokine **18**, 61 (2002)
- (6) Innate immunity mediated by the cytokine IL-1 homologue 4 (IL-1H4/IL-1F7) induces IL-12-dependent adaptive and profound antitumor immunity: W. Gao, et al.; J. Immunol. **170**, 107 (2003)

4. Assay Principle

This assay is a sandwich Enzyme Linked-Immunosorbent Assay (ELISA) for quantitative determination of human IL-37 in biological fluids. A polyclonal antibody specific for IL-37 has been precoated onto the 96-well microtiter plate. Standards and samples are pipetted into the wells for binding to the coated antibody. After extensive washing to remove unbound compounds, IL-37 is recognized by the addition of a polyclonal antibody specific for IL-37 (Detection Antibody). After removal of excess polyclonal antibody, HRP conjugated anti-rabbit IgG (HRP) 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 IL-37 in the samples.

5. Handling & Storage

- Reagent must be stored at 2-8°C when not in use.
- Plate and reagents should be at room temperature before use.
- Do not expose reagents to temperatures greater than 25°C.

6. Kit Components

1 plate coated with human IL-37 Antibody	(6 x 16-well strips)	
2 bottles Wash Buffer 10X	(2 x 30 ml)	(Wash Buffer 10X)
2 bottles ELISA Buffer 10X	(2 x 30 ml)	(ELISA Buffer 10X)
1 vial Detection Antibody	(30 µl)	(DET)
1 vial HRP 100X (HRP Conjugated anti-rabbit IgG)	(150 µl)	(HRP 100X)
1 vial human IL-37 Standard (lyophilized)	(2 ng)	(STD)
1 bottle TMB Substrate Solution	(12 ml)	(TMB)
1 bottle Stop Solution	(12 ml)	(STOP)
2 plate sealers (plastic film)		
2 silica Gel Minibags		

7. Materials Required but *Not* Supplied

- Microtiterplate reader at 450 nm
- Calibrated precision single and multi-channel pipettes. Disposable pipette tips
- Deionized water
- Microtubes or equivalent for preparing dilutions
- Disposable plastic containers for preparing working buffers
- Plate washer: automated or manual
- Glass or plastic tubes for diluting and aliquoting standard

8. General ELISA Protocol

8.1. Preparation and Storage of Reagents

NOTE: Prepare just the appropriate amount of the buffers necessary for the assay.

- **Wash Buffer 10X** has to be diluted with deionized water 1:10 before use (e.g. 50 ml Wash Buffer 10X + 450 ml water) to obtain Wash Buffer 1X.
- **ELISA Buffer 10X** has to be diluted with deionized water 1:10 before use (e.g. 20 ml ELISA Buffer 10X + 180 ml water) to obtain ELISA Buffer 1X.
- **Detection Antibody (DET)** has to be diluted to 1:1000 in ELISA Buffer 1X (10 µl DET + 10 ml ELISA Buffer 1X).

NOTE: The diluted Detection Antibody is not stable and cannot be stored!

- **HRP 100X (HRP Conjugated anti-rabbit IgG)** has to be diluted to the working concentration by adding 100 µl in 10 ml of ELISA Buffer 1X (1:100).

NOTE: The diluted HRP is used within one hour of preparation.

- **Human IL-37 Standard (STD)** has to be reconstituted with 1 ml of deionized water.
 - This reconstitution produces a stock solution of 2 ng/ml. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes. Mix well prior to making dilutions.

NOTE: The reconstituted standard is aliquoted and stored at -20°C.

- Dilute the standard protein concentrate (STD) (**2 ng/ml**) in ELISA Buffer 1X. A seven-point standard curve using 2-fold serial dilutions in ELISA Buffer 1X is recommended.
- Suggested standard points are:
1 , 0.5 , 0.25 , 0.125 , 0.063 , 0.031 , 0.016 and 0 ng/ml.

Dilute further for the standard curve:

To obtain	Add	Into
1 ng/ml	300 µl of IL-37 (2 ng/ml)	300 µl of ELISA Buffer 1X
0.5 ng/ml	300 µl of IL-37 (1 ng/ml)	300 µl of ELISA Buffer 1X
0.25 ng/ml	300 µl of IL-37 (0.5 ng/ml)	300 µl of ELISA Buffer 1X
0.125 ng/ml	300 µl of IL-37 (0.25 ng/ml)	300 µl of ELISA Buffer 1X
0.063 ng/ml	300 µl of IL-37 (0.125 ng/ml)	300 µl of ELISA Buffer 1X
0.031 ng/ml	300 µl of IL-37 (0.063 ng/ml)	300 µl of ELISA Buffer 1X
0.016 ng/ml	300 µl of IL-37 (0.031 ng/ml)	300 µl of ELISA Buffer 1X
0 ng/ml	300 µl of ELISA Buffer 1X	Empty tube

8.2. Sample Collection, storage and dilution

Serum : Use a serum separator tube. Let samples clot at room temperature for 30 minutes before centrifugation for 20 minutes at 1,000xg. Assay freshly prepared serum or store serum in aliquot at $\leq -20^{\circ}\text{C}$ for later use. Avoid repeated freeze/thaw cycles.

Plasma : Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. Assay freshly prepared plasma or store plasma sample in aliquot at $\leq -20^{\circ}\text{C}$ for later use. Avoid repeated freeze/ thaw cycles.

Serum or **Plasma** have to be diluted in ELISA Buffer 1X. Samples containing visible precipitates must be clarified before use.

NOTE: As a starting point, 1/2 dilution of serum and plasma is recommended! If sample values fall outside the detection range of the assay, a lower or higher dilution may be required!

8.3. Assay Procedure (Checklist)

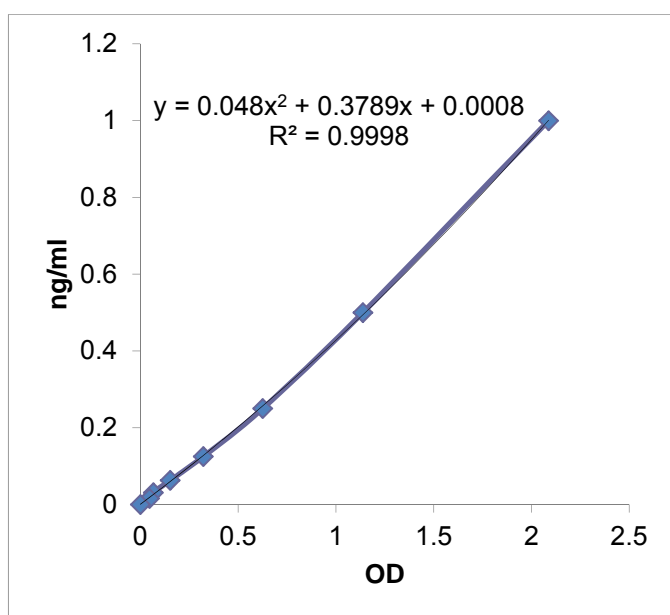
<input type="checkbox"/>	<p>1. Determine the number of 16-well strips needed for the assay and insert them in the frame for current use. The extra strips should be resealed in the foil pouch bag and stored at 4°C.</p> <p>NOTE: Remaining 16-well strips coated with IL-37 antibody when opened can be stored at 4°C for up to 1 month.</p>
<input type="checkbox"/>	<p>2. Add 100 µl of the different standards into the appropriate wells in duplicate! At the same time, add 100 µl of diluted serum or plasma samples in duplicate to the wells (see 8.1. Preparation and Storage of Reagents and 8.2. Preparation of Samples).</p>
<input type="checkbox"/>	<p>3. Cover the plate with plate sealer and incubate for overnight at 4°C.</p>
<input type="checkbox"/>	<p>4. Aspirate the coated wells and add 300 µl of Wash Buffer 1X using a multichannel pipette or auto-washer. Repeat the process for a total of three washes. After the last wash, complete removal of liquid is essential for good performance.</p>
<input type="checkbox"/>	<p>5. Add 100 µl to each well of the Detection Antibody (DET) (see 8.1. Preparation and Storage of Reagents).</p>
<input type="checkbox"/>	<p>6. Cover the plate with plate sealer and incubate for 1 hour at 37°C.</p>
<input type="checkbox"/>	<p>7. Aspirate the coated wells and add 300 µl of Wash Buffer 1X using a multichannel pipette or auto-washer. Repeat the process for a total of three washes. After the last wash, complete removal of liquid is essential for good performance.</p>
<input type="checkbox"/>	<p>8. Add 100 µl to each well of the diluted HRP (see 8.1. Preparation and Storage of Reagents).</p>
<input type="checkbox"/>	<p>9. Cover the plate with plate sealer and incubate for 1 hour at 37°C.</p>
<input type="checkbox"/>	<p>10. Aspirate the coated wells and add 300 µl of Wash Buffer 1X using a multichannel pipette or auto-washer. Repeat the process for a total of five washes. After the last wash, complete removal of liquid is essential for good performance.</p>
<input type="checkbox"/>	<p>11. Add 100 µl to each well of TMB Substrate Solution (TMB).</p>
<input type="checkbox"/>	<p>12. Allow the color reaction to develop at room temperature (RT°C) in the dark for 10 minutes.</p>
<input type="checkbox"/>	<p>13. Stop the reaction by adding 100 µl of Stop Solution (STOP). Tap the plate gently to ensure thorough mixing. The substrate reaction yields a blue solution that turns yellow when Stop Solution is added.</p>
	<p>! CAUTION: CORROSIVE SOLUTION!</p>
<input type="checkbox"/>	<p>14. Measure the OD at 450 nm in an ELISA reader within 30 minutes.</p>

9. Calculation of Results

- Average the duplicate readings for each standard, control and sample and subtract the average blank value (obtained with the 0 ng/ml point).
- Generate the standard curve by plotting the average absorbance obtained for each standard concentration on the horizontal (X) axis vs. the corresponding IL-37 concentration (ng/ml) on the vertical (Y) axis (see 10. TYPICAL DATA).
- Calculate the IL-37 concentrations of samples by interpolation of the regression curve formula as shown above in a form of a quadratic equation.
- If the test samples were diluted, multiply the interpolated values by the dilution factor to calculate the concentration of human IL-37 in the samples.

10. Typical Data

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



Standard hIL-37 (ng/ml)	Optical Density (mean)
1	2.0878
0.5	1.138
0.25	0.6258
0.125	0.3222
0.063	0.1526
0.031	0.0668
0.016	0.0466
0	0

Figure: Standard curve

11. Performance Characteristics

A. Sensitivity (Limit of detection):

The lowest level of IL-37 that can be detected by this assay is 10 pg/ml. **NOTE:** *The Limit of detection was measured by adding two standard deviations to the mean value of 50 zero standard.*

B. Assay range: 0.016 ng/ml – 1 ng/ml

C. Specificity:

This ELISA is specific for the measurement of natural and recombinant human IL-37. It does not cross-react with human IL-33, human IL-6, human IL-23, human IL-24, human adiponectin, human vaspin, human clusterin, human GPX3, human ANGPTL4, human RBP4, human FTO, human Sirtuin2, human calreticulin, human FABP4, human CTRP5, mouse IL-33, mouse adiponectin, rat adiponectin, rat Nampt.

D. Intra-assay precision:

Five samples of known concentrations of human IL-37 were assayed in replicates 4 times to test precision within an assay.

Sample	Means (ng/ml)	SD	CV (%)	n
1	0.085	0.007	8.542	4
2	0.222	0.019	8.655	4
3	0.250	0.011	4.210	4
4	0.047	0.004	9.446	4
5	0.092	0.003	3.509	4

E. Inter-assay precision:

Six samples of known concentrations of human IL-37 were assayed in 6 separate assays to test precision between assays.

Sample	Means (ng/ml)	SD	CV (%)	n
1	0.093	0.005	4.948	6
2	0.214	0.020	9.455	6
3	0.261	0.015	5.896	6
4	0.056	0.005	8.100	6
5	0.093	0.007	6.992	6
6	0.208	0.019	9.015	6

F. Linearity:

Different human serum samples containing IL-37 were diluted several fold (1 to 1/2) and the measured recoveries ranged from 90% to 110%.

Sample	Sample Dilution	Expected (ng/ml)	Observed (ng/ml)	% of Expected
1	1	0.096	0.096	100
	1 : 2	0.048	0.052	108.16
2	1	0.036	0.036	100
	1 : 2	0.018	0.018	100.26
3	1	0.050	0.050	100
	1 : 2	0.025	0.024	93.53

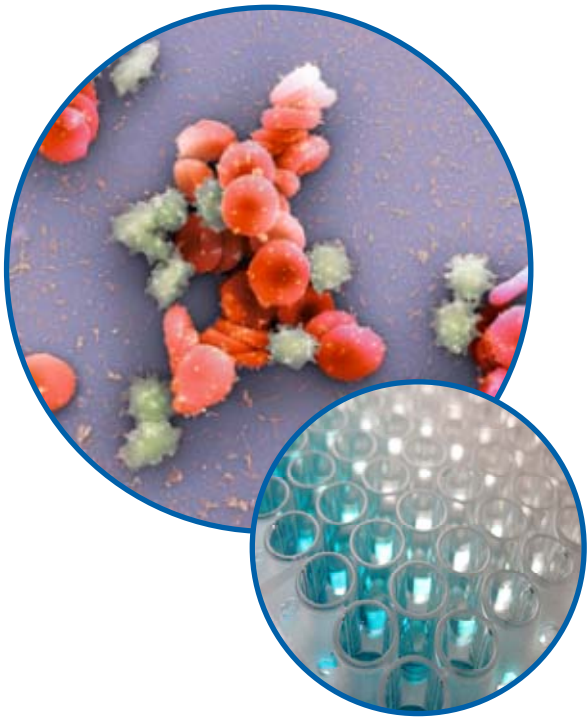
12. Technical Hints and Limitations

- It is recommended that all standards, controls and samples be run in duplicate.
- Do not combine leftover reagents with those reserved for additional wells.
- Reagents from the kit with a volume less than 100 µl should be centrifuged.
- Residual wash liquid should be drained from the wells after last wash by tapping the plate on absorbent paper.
- Crystals could appear in the 10X solution due to high salt concentration in the stock solutions. Crystals are readily dissolved at room temperature or at 37°C before dilution of the buffer solutions.
- Once reagents have been added to the 16-well strips, DO NOT let the strips DRY at any time during the assay.
- Keep TMB Substrate Solution (TMB) protected from light.
- The Stop Solution (STOP) consists of sulfuric acid. Although diluted, the Stop Solution (STOP) should be handled with gloves, eye protection and protective clothing.

13. Troubleshooting

PROBLEM	POSSIBLE CAUSES	SOLUTIONS
No signal or weak signal	Omission of key reagent	Check that all reagents have been added in the correct order.
	Washes too stringent	Use an automated plate washer if possible.
	Incubation times inadequate	Incubation times should be followed as indicated in the manual.
	Plate reader settings not optimal	Verify the wavelength and filter setting in the plate reader.
	Incorrect assay temperature	Use recommended incubation temperature. Bring substrates to room temperature before use.
High background	Concentration of HRP too high	Use recommended dilution factor.
	Inadequate washing	Ensure all wells are filling wash buffer and are aspirated completely.
Poor standard curve	Wells not completely aspirated	Completely aspirate wells between steps.
	Reagents poorly mixed	Be sure that reagents are thoroughly mixed.
Unexpected results	Omission of reagents	Be sure that reagents were prepared correctly and added in the correct order.
	Dilution error	Check pipetting technique and double-check calculations.

14. Notes



Product Specific References:

1. C. Li, et al.; J. Interferon Cytokine Res. **33**, 612 (2013)
2. C. Li, et al.; Mediators Inflamm. **2013**, 639712 (2013)
3. L. Ye, et al.; J. Transl. Med. **12**, 69 (2014)
4. P.-W. Zhao, et al.; PLOS One **9**, e95346 (2014)
5. P. Italiani, et al.; J. Neuroinflamm. **11**, 94 (2014)

For more References please visit www.adipogen.com!

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