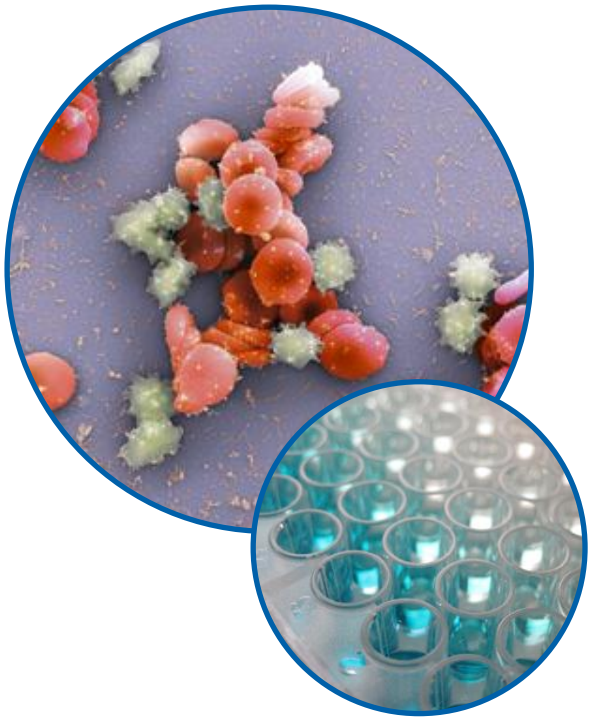


AdipoGen™

MANUAL

IL-37 (human) (IntraCellular) ELISA Kit

For research use only. Not for diagnostic use.

Version 1 (29-April-2011)

Cat. No. AG-45A-0040EK-KI01

Table of Contents

1. Intended Use

The IL-37 (human) (IntraCellular) ELISA Kit is to be used for the *in vitro* quantitative determination of human IL-37 in cell lysates or cell-based assays (screening). 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 NFKB 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-meidated 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.; Proc. Natl. Acad. Sci. 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 cells. 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 purified polyclonal antibody specific for IL-37 (Detection Antibody). After removal of excess polyclonal antibody, HRP conjugated anti-rabbit IgG (Detector) 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	(12 x 8-well strips)
1 bottle Wash Buffer 10X	(50 ml)
1 bottle Diluent 5X	(50 ml)
1 bottle Lysis Buffer 10X	(12 ml)
1 bottle Detection Antibody	(12 ml)
1 vial Detector 100X (HRP Conjugated anti-rabbit IgG)	(150 µl)
1 vial human IL-37 Standard (lyophilized)	(4 ng)
1 vial human IL-37 QC sample (lyophilized)	
1 bottle Substrate Solution I (TMB)	(6 ml)
1 bottle Substrate Solution II (Peroxidase)	(6 ml)
1 bottle Stop Solution	(12 ml)
3 plate sealers (plastic film)	

7. Materials Required but *Not* Supplied

- Microtiterplate reader at 450 nm, with the correction wavelength set at 540 nm or 570 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
- Phenyl methylsulfonyl fluoride (PMSF)

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.
- **Diluent 5X** has to be diluted with deionized water 1:5 before use (e.g. 50 ml Diluent 5X + 200 ml water) to obtain Diluent 1X.
- **Lysis Buffer 10X** has to be diluted with deionized water 1:10 before use (e.g. 12 ml Lysis Buffer 10X + 108 ml water) to obtain Lysis Buffer 1X. Add 1 mM PMSF immediately before use.
- **Detector 100X (HRP Conjugated anti-rabbit IgG)** has to be diluted to the working concentration by adding 120 µl in 12 ml of Diluent 1X (1:100).

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

- **Substrate Solution I and II** have to be mixed together in equal volumes within 15 minutes of use.

  NOTE: *Freshly prepare just before use the Substrate Solution and protect from light!*

- **Human IL-37 Standard (STD)** has to be reconstituted with 1 ml of deionized water.

  - This reconstitution produces a stock solution of 4 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) (**4 ng/ml**) in Diluent 1X. A seven-point standard curve using 2-fold serial dilutions in Diluent 1X is recommended.
  - Suggested standard points are:

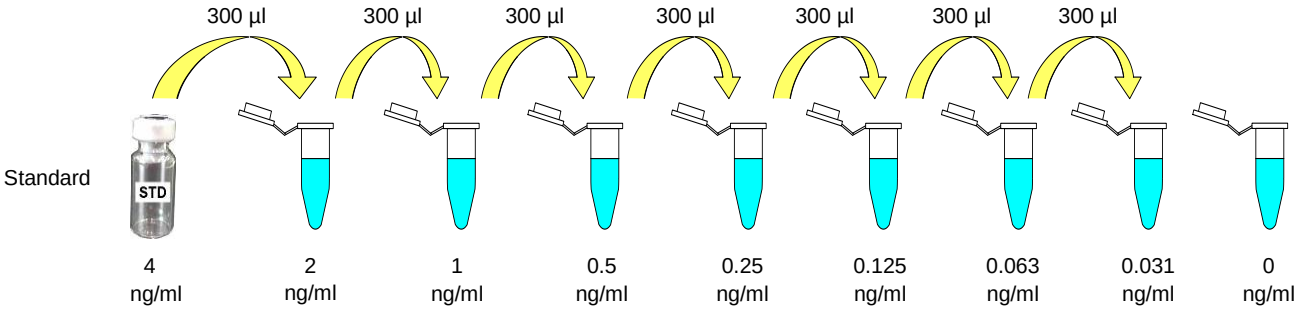
    2 , 1 , 0.5 , 0.25 , 0.125 , 0.063 , 0.031 and 0 ng/ml.

- **Human IL-37 QC sample** has to be reconstituted with 1 ml of deionized water.

  - Refer to the Certificate of Analysis for current QC sample concentration. Mix the QC sample to ensure complete reconstitution and allow the QC sample to sit for a minimum of 15 minutes. The reconstituted QC sample is ready to use, do not dilute it.

Dilute further for the standard curve:

To obtain	Add	Into
2 ng/ml	300 µl of IL-37 (4 ng/ml)	300 µl of Diluent 1X
1 ng/ml	300 µl of IL-37 (2 ng/ml)	300 µl of Diluent 1X
0.5 ng/ml	300 µl of IL-37 (1 ng/ml)	300 µl of Diluent 1X
0.25 ng/ml	300 µl of IL-37 (0.5 ng/ml)	300 µl of Diluent 1X
0.125 ng/ml	300 µl of IL-37 (0.25 ng/ml)	300 µl of Diluent 1X
0.063 ng/ml	300 µl of IL-37 (0.125 ng/ml)	300 µl of Diluent 1X
0.031 ng/ml	300 µl of IL-37 (0.063 ng/ml)	300 µl of Diluent 1X
0 ng/ml	300 µl of Diluent 1X	Empty tube



8.2. Sample Collection, Storage and Dilution

Cell Lysates : Grow cell until 90% confluency. Scrap cells off the plate and transfer to an appropriate tube. Keep on ice and microcentrifuge at 1,200 rpm for 5 minutes at 4°C. Remove supernatant, rinse cells once with ice-cold PBS. Remove PBS and add 200 µl ice-cold 1x lysis buffer supplemented with 1 mM phenyl methylsulfonyl fluoride (PMSF) to ten million cells of interest and incubate on ice for 30 minutes. Microcentrifuge at 12,000 rpm for 5 minutes at 4°C and transfer the supernatant to a new tube. The supernatant is the cell lysate. Use freshly prepared cell lysate samples.

Cell Lysates have to be diluted in Diluent 1X. Samples containing visible precipitates must be clarified before use.

NOTE: *As a starting point, 1 to 1/1,000 dilutions of cell lysates are recommended! If samples fall the outside range of assay, a lower or higher dilution may be required!*

8.3. Assay Procedure (Checklist)

☐	1. Determine the number of 8-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. ***NOTE:*** *Remaining 8-well strips coated with IL-37 antibody when opened can be stored at 4°C for up to 1 month.*
☐	2. Add 100 µl of the different standards into the appropriate wells in duplicate! At the same time, add 100 µl of diluted lysates samples in duplicate to the wells **(see 8.1. Preparation and Storage of Reagents and 8.2. Preparation of Samples).**
☐	3. Cover the plate with plate sealer and incubate for **1 hour at 37°C.**
☐	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.
☐	5. Add 100 µl to each well of the Detection Antibody.
☐	6. Cover the plate with plate sealer and incubate for **1 hour at 37°C**.
☐	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.
☐	8. Add 100 µl to each well of the diluted Detector **(see 8.1. Preparation and Storage of Reagents)**.
☐	9. Cover the plate with plate sealer and incubate for **1 hour at 37°C**.
☐	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.
☐	11. Add 100 µl to each well of mixed substrate solution.
☐	12. Allow the color reaction to develop **at room temperature (RT°C) in the dark for 10 minutes**.
☐	13. Stop the reaction by adding 100 µl of Stop Solution. Tap the plate gently to ensure thorough mixing. The substrate reaction yields a blue solution that turns yellow when Stop Solution is added.
	! CAUTION: CORROSIVE SOLUTION!
☐	14. Measure the OD at 450 nm in an ELISA reader within 30 minutes.

9. Calculation of Results

- Average the duplicate readings for each standard, QC 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:

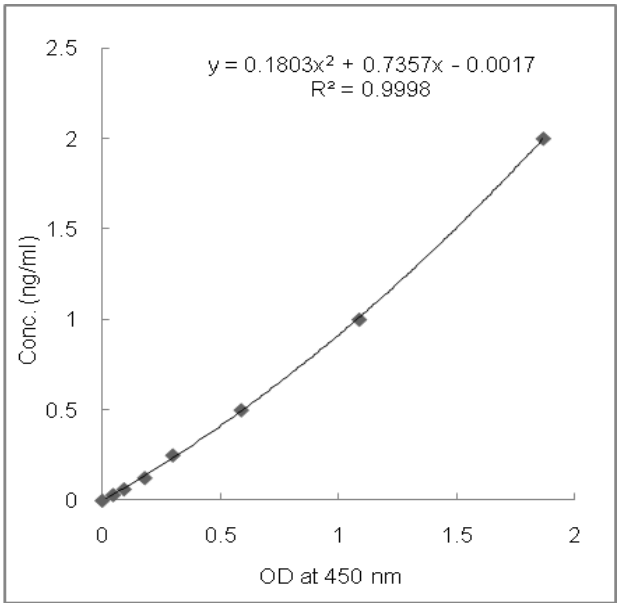


Figure: Standard curve

Standard hIL-37 (ng/ml)	Optical Density (mean)
2	1.865
1	1.086
0.5	0.588
0.25	0.298
0.125	0.180
0.063	0.092
0.031	0.046
0	0

11. Performance Characteristics

A. Sensitivity (Limit of detection):

The lowest level of IL-37 that can be detected by this assay is 20 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: 0.031 ng/ml – 2 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 pro IL-33, human IL-33 (homeodomain-like helix tern helix), human IL-23, human IL-17A, human IL-6, human IL-24, human IL-23p19, human IL-12p40, human adiponecitn, human vaspin, human sirtuin1, human sirtuin2, human FTO, human ST2, human CTRP5, human FABP4, human ANGPTL3, human DNER, human calreticulin, mouse IL-33, mouse adiponectin, rat adiponectin.

D. Intra-assay precision:

Six cell lysates of known concentrations of human IL-37 were assayed in replicates 12 times to test precision within an assay.

Samples	Means (ng/ml)	SD	CV (%)	n
1	664.86	8.09	1.22	12
2	809.14	16.53	2.04	12
3	389.54	23.77	6.10	12
4	321.70	9.69	3.01	12
5	261.73	17.42	6.65	12
6	209.21	11.62	5.56	12

E. Inter-assay precision:

Six cell lysates of known concentrations of human IL-37 were assayed in 5 separate assays to test precision between assays.

Samples	Means (ng/ml)	SD	CV (%)	n
1	656.81	11.90	1.81	5
2	737.67	62.58	8.48	5
3	437.06	14.12	3.23	5
4	331.12	5.87	1.77	5
5	267.09	6.51	2.44	5
6	227.31	15.07	6.63	5

F. Recovery:

When samples (cell lysates) are spiked with known concentrations of human IL-37, the recovery averages 100% (range from 90% to 105%).

Samples	Average recovery (%)	Range (%)
1	100.94	95-105
2	100.54	95-105
3	102.24	95-105
4	99.43	95-105
5	99.59	95-105
6	98.58	90-105

G. Linearity:

Different human cell lysates containing IL-37 were diluted several fold (1/800 to 1/3,200) and the measured recoveries ranged from 95% to 105%.

Samples	Sample Dilution	Expected (ng/ml)	Observed (ng/ml)	% of Expected
1	1 : 800	564.96	564.96	100
	1 : 1,600	282.48	283.73	100.44
	1 : 3,200	141.24	138.53	98.08
2	1 : 800	629.16	629.16	100
	1 : 1,600	314.58	325.27	103.40
	1 : 3,200	157.29	163.21	103.76

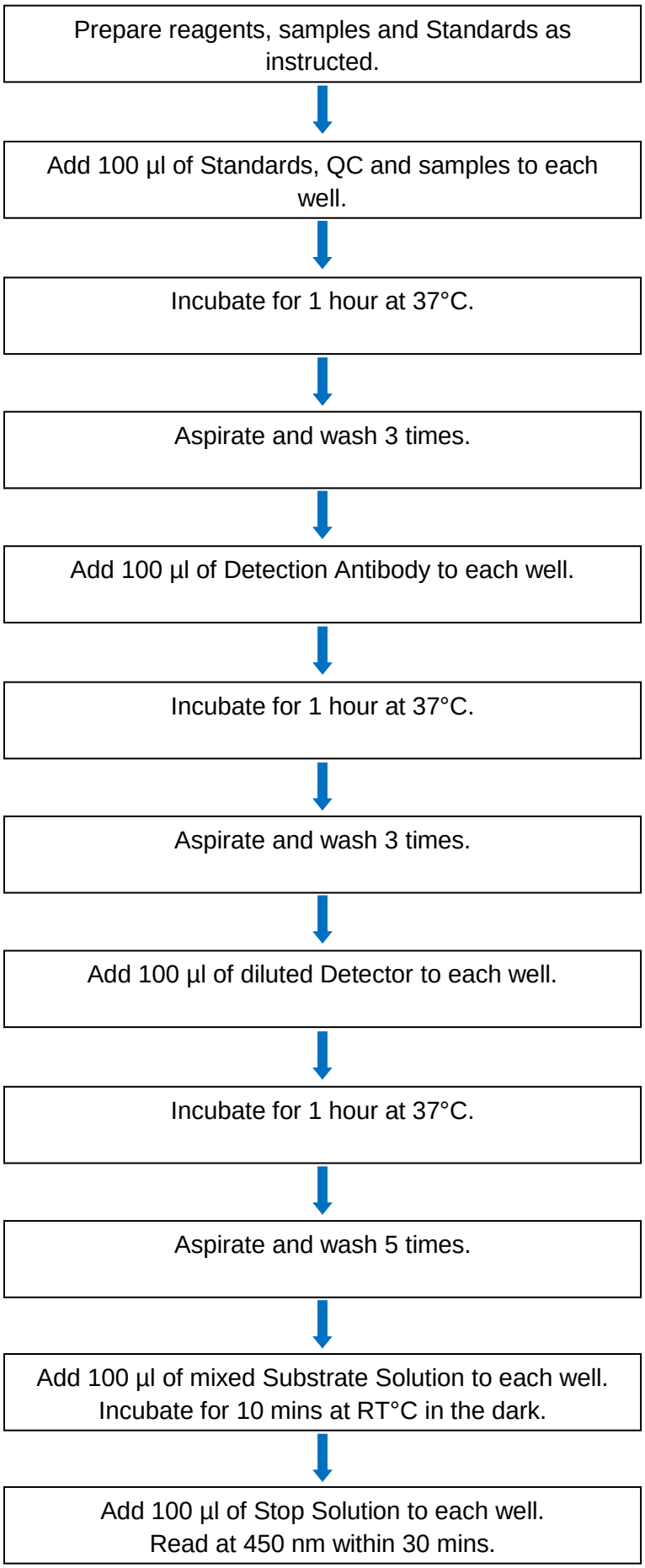
12. Technical Hints and Limitations

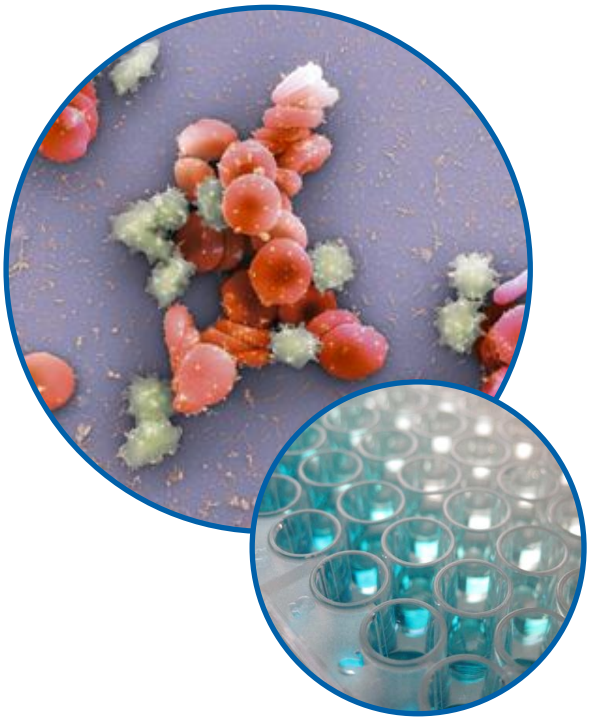
- It is recommended that all standards, QC sample and samples be run in duplicate.
- Do not combine leftover reagents with those reserved for additional wells.
- Reagents from the kit with a volume less than 100 µl should be centrifuged.
- Residual wash liquid should be drained from the wells after last 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 8-well strips, DO NOT let the strips DRY at any time during the assay.
- Keep Substrate Solution protected from light.
- The Stop Solution consists of phosphoric acid. Although diluted, the Stop Solution 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 detector 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. Assay Flow Chart





Product Specific References:

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