ab174445 – ICAM-1 (CD54) Human SimpleStep ELISA® Kit

Instructions for Use

For the quantitative measurement of ICAM1 in human cell culture supernatant, plasma, serum and cell extracts.

This product is for research use only and is not intended for diagnostic use.

Version 3 Last Updated 13 January 2022

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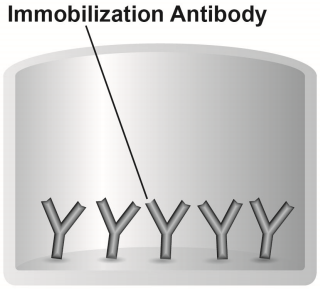
1. BACKGROUND

ICAM-1 (CD54) *in vitro* SimpleStep ELISA® (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of ICAM-1 protein in human cell culture supernatant, plasma, serum and cell extracts.

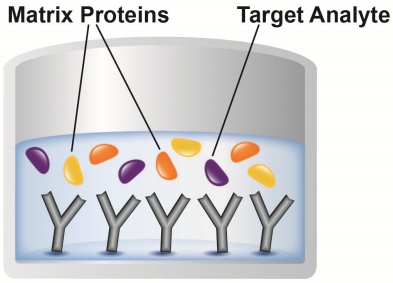
The SimpleStep ELISA® employs an affinity tag labeled capture antibody and a reporter conjugated detector antibody which immunocapture the sample analyte in solution. This entire complex (capture antibody/analyte/detector antibody) is in turn immobilized via immunoaffinity of an anti-tag antibody coating the well. To perform the assay, samples or standards are added to the wells, followed by the antibody mix. After incubation, the wells are washed to remove unbound material. TMB Development Solution is added and during incubation is catalyzed by HRP, generating blue coloration. This reaction is then stopped by addition of Stop Solution completing any color change from blue to yellow. Signal is generated proportionally to the amount of bound analyte and the intensity is measured at 450 nm. Optionally, instead of the endpoint reading, development of TMB can be recorded kinetically at 600 nm.

ICAM1, is a cell surface glycoprotein typically expressed in endothelial cells and cells of the immune system. The extracellular portion of ICAM-1 forms five immunoglobulin like domains attached to a single hydrophobic transmembrane region and a short cytoplasmic tail. ICAM-1, binds to the Leukocyte Integrins LFA-1 (CD11a/CD18) and Mac-1 (CD11b/CD18) as well as to non integrin ligands such as CD43, fibrinogen, hyaluronan, Rhinoviruses and *Plasmodium falciparum*-infected erythrocytes. Binding to LFA-1 facilitates trans-endothelial leukocyte migration to areas of inflammation via promotion of endothelial apical cups assembly.

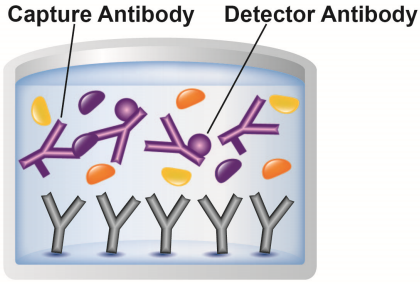
2. ASSAY SUMMARY



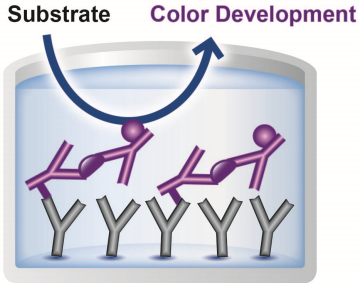
Remove appropriate number of antibody coated well strips. Equilibrate all reagents to room temperature. Prepare all reagents, samples, and standards as instructed.



Add standard or sample to appropriate wells.



Add Antibody Cocktail to all wells. Incubate at room temperature.



Aspirate and wash each well. Add TMB Development Solution to each well and incubate. Add Stop Solution at a defined endpoint.

Alternatively, record color development kinetically after TMB substrate addition.

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at 2-8ºC immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in Reagent and Standard preparation sections.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
10X ICAM-1 Capture Antibody	600 µL	+2-8ºC
10X ICAM-1 Detector Antibody	600 µL	+2-8ºC
ICAM-1 Human Lyophilized Recombinant Protein	2 Vials	+2-8ºC
Antibody Diluent 4BI	6 mL	+2-8ºC
10X Wash Buffer PT	20 mL	+2-8ºC
5X Cell Extraction Buffer PTR	10 mL	+2-8ºC
50X Cell Extraction Enhancer Solution	1 mL	+2-8ºC
TMB Development Solution	12 mL	+2-8ºC
Stop Solution	12 mL	+2-8ºC
Sample Diluent NS	50 mL	+2-8ºC
Pre-Coated 96 Well Microplate (12 x 8 well strips)	96 Wells	+2-8ºC
Plate Seal	1	+2-8ºC

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 or 600 nm.
- Method for determining protein concentration (BCA assay recommended).
- Deionized water.
- PBS (1.4 mM KH2PO4, 8 mM Na2HPO4, 140 mM NaCl, 2.7 mM KCl, pH 7.4).
- Multi- and single-channel pipettes.
- Tubes for standard dilution.
- Plate shaker for all incubation steps.
- Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

8. TECHNICAL HINTS

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.
- As a guide, typical ranges of sample concentration for commonly used sample types are shown below in Sample Preparation (section 11).
- All samples should be mixed thoroughly and gently.
- Avoid multiple freeze/thaw of samples.
- Incubate ELISA plates on a plate shaker during all incubation steps.
- When generating positive control samples, it is advisable to change pipette tips after each step.
- The provided 5X Cell Extraction Buffer contains phosphatase inhibitors. Protease inhibitors can be added if required.
- The provided 50X Cell Extraction Enhancer Solution may precipitate when stored at + 4ºC. To dissolve, warm briefly at + 37ºC and mix gently. The 50X Cell Extraction Enhancer Solution can be stored at room temperature to avoid precipitation.
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**

9. REAGENT PREPARATION

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. **The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.**
- Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations.

9.1 1X Cell Extraction Buffer PTR (For cell and tissue extracts only)

If required, prepare 1X Cell Extraction Buffer PTR by diluting 5X Cell Extraction Buffer PTR and 50X Cell Extraction Enhancer Solution to 1X with deionized water. To make 10 mL 1X Cell Extraction Buffer PTR combine 7.8 mL deionized water, 2 mL 5X Cell Extraction Buffer PTR and 200 µL 50X Cell Extraction Enhancer Solution Mix thoroughly and gently. If required protease inhibitors can be added.

Alternative – Enhancer may be added to Cell Extraction Buffer after extraction of cells or tissue. Refer to note in Troubleshooting section.

9.2 1X Wash Buffer PT

Prepare 1X Wash Buffer PT by diluting 10X Wash Buffer PT with deionized water. To make 50 mL 1X Wash Buffer PT combine 5 mL 10X Wash Buffer PT with 45 mL deionized water. Mix thoroughly and gently.

9.3 Antibody Cocktail

Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent 4BI. To make 3 mL of the Antibody Cocktail combine 300 µL 10X Capture Antibody and 300 µL 10X Detector Antibody with 2.4 mL Antibody Diluent 4BI. Mix thoroughly and gently.

10. STANDARD PREPARATION

Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of positive controls for every use.

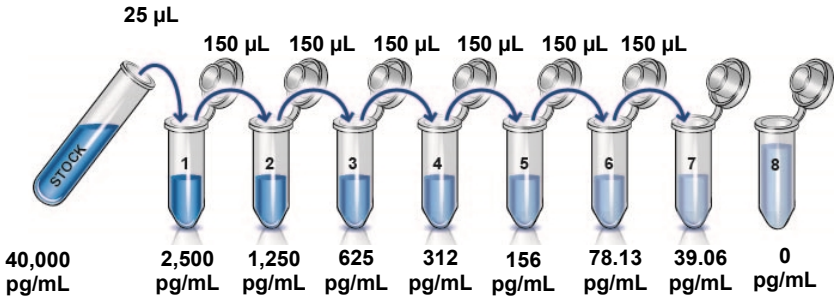
The following section describes the preparation of a standard curve for duplicate measurements (recommended).

10.1 IMPORTANT: If the protein standard vial has a volume identified on the label, reconstitute the ICAM-1 standard by adding that volume of Sample Diluent NS indicated on the label. Alternatively, if the vial has a mass identified, reconstitute the ICAM-1 standard by adding 500 µL Sample Diluent NS. Hold at room temperature for 3 minutes and mix gently. Ensure all protein is reconstituted by inspecting against the light. This is the 40,000 pg/mL **Stock Standard** Solution.

10.2 Label eight tubes, Standards 1– 8.

10.3 Add 375 µL of Sample Diluent NS to tube #1 and 150 µL of Sample Diluent NS to tubes #2-8.

10.4 Use the Stock Standard to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:



11. SAMPLE PREPARATION

TYPICAL SAMPLE DYNAMIC RANGE	
Sample Type	**Loading Range**
48 hours PHA-stimulated PBMC supernatant	3 – 50%
Human Serum	0.03 – 0.5%
Human Plasma - Heparin	0.03 – 0.5%
Human Plasma - EDTA	0.03 – 0.5%
Human Plasma - Citrate	0.03 – 0.5%
Raji Cell Lysate	0.1 – 5 µg/mL
HepG2 Cell Lysate	0.6 – 10 µg/mL
HeLa Cell Lysate	2 – 50 µg/mL

11.1 **Plasma**

Collect plasma using citrate, EDTA or heparin. Centrifuge samples at 2,000 x g for 10 minutes. Samples **MUST** be diluted at least 100X or more in Sample Diluent NS to prevent saturation of the signal. Store un-diluted plasma samples at -20ºC or below for up to 3 months. Avoid repeated freeze-thaw cycles.

11.2 **Serum**

Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2,000 x g for 10 minutes and collect serum. Samples **MUST** be diluted at least 100X or more in Sample Diluent NS to prevent saturation of the signal. Store un-diluted serum at -20ºC or below. Avoid repeated freeze-thaw cycles.

11.3 Cell Culture Supernatants

Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Cell culture supernatants should be diluted at least two-fold in Sample Diluent NS. Undiluted samples should be stored at -20°C or below. Avoid repeated freeze-thaw cycles.

11.4 Preparation of extracts from cell pellets

11.1.1 Collect non-adherent cells by centrifugation or scrape to collect adherent cells from the culture flask. Typical centrifugation conditions for cells are 500 x g for 5 minutes at 4ºC.

11.1.2 Rinse cells twice with PBS.

11.1.3 Solubilize pellet at 2x107 cell/mL in chilled 1X Cell Extraction Buffer PTR.

11.1.4 Incubate on ice for 20 minutes.

11.1.5 Centrifuge at 18,000 x g for 20 minutes at 4°C.

11.1.6 Transfer the supernatants into clean tubes and discard the pellets.

11.1.7 Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

11.1.8 Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

12. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.
- For each assay performed, a minimum of two wells must be used as the zero control.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Differences in well absorbance or “edge effects” have not been observed with this assay.

13. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**

13.1 Prepare all reagents, working standards, and samples as directed in the previous sections.

13.2 Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4ºC storage.

13.3 Add 50 µL of all samples and standards to appropriate wells.

13.4 Add 50 µL of the Antibody Cocktail to each well.

13.5 Seal or cover plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm.

13.6 Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and blot it against clean paper towels to remove excess liquid.

13.7 Add 100 µL of TMB Development Solution to each well and incubate for 10 minutes in the dark on a plate shaker set to 400 rpm.

Given variability in laboratory environmental conditions, optimal incubation time may vary between 5 and 20 minutes.

Note: The addition of Stop Solution will change the color from blue to yellow and enhance the signal intensity about 3X. To avoid signal saturation, proceed to the next step before the high concentration of the standard reaches a blue color of O.D.600 equal to 1.0.

13.8 Add 100 µL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading.

Alternative to 13.7 – 13.8: Instead of the endpoint reading at 450 nm, record the development of TMB Substrate kinetically. Immediately after addition of TMB Development Solution begin recording the blue color development with elapsed time in the microplate reader prepared with the following settings:

Mode:	Kinetic
Wavelength:	600 nm
Time:	up to 20 min
Interval:	20 sec - 1 min
Shaking:	Shake between readings

Note that an endpoint reading can also be recorded at the completion of the kinetic read by adding 100 µL Stop Solution to each well and recording the OD at 450 nm.

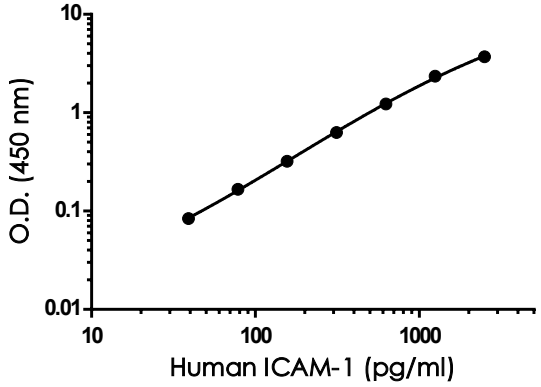
13.9 Analyze the data as described below.

14. CALCULATIONS

Subtract average zero standard from all readings. Average the duplicate readings of the positive control dilutions and plot against their concentrations. Draw the best smooth curve through these points to construct a standard curve. Most plate reader software or graphing software can plot these values and curve fit. A four-parameter algorithm (4PL) usually provides the best fit, though other equations can be examined to see which provides the most accurate (e.g. linear, semi-log, log/log, 4 parameter logistic). Interpolate protein concentrations for unknown samples from the standard curve plotted. Samples producing signals greater than that of the highest standard should be further diluted and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.

15. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.



Standard Curve Measurements			
Conc. (pg/mL)	O.D 450 nm		Mean O.D
	1	2	
0	0.042	0.046	0.044
39.1	0.129	0.127	0.128
78.1	0.211	0.209	0.210
156.2	0.360	0.368	0.364
312.5	0.661	0.683	0.672
625	1.274	1.268	1.271
1,250	2.342	2.446	2.394
2,500	3.730	3.765	3.748

Figure 1. Example of ICAM-1 standard curve. The ICAM-1 standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

16. TYPICAL SAMPLE VALUES

SENSITIVITY –

The calculated minimal detectable (MDD) dose is 1.6 pg/mL. The MDD was determined by calculating the mean of zero standard replicates (n=16) and adding 2 standard deviations then extrapolating the corresponding concentrations.

RECOVERY –

(Sample spiking in representative sample matrices)

Sample Type	Average % Recovery	Range (%)
50% Culture Media	98	91 - 102
50% Extraction Buffer	87	82 – 97
1% Non reactive Serum	103	99 - 110
1% Non reactive Plasma	113	96 - 126

LINEARITY OF DILUTION –

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Dilution Factor	Interpolated value	1% Serum	1% Plasma Citrate	1% Plasma EDTA	1% Plasma Heparin	50% Cell Media
Undiluted	pg/mL	3,804	4,632	4,135	4,818	944
	% Expected value	**100**	**100**	**100**	**100**	**100**
2	pg/mL	1,767	2,342	2,180	2,602	455
	% Expected value	**93**	**101**	**105**	**108**	**91**
4	pg/mL	969	1,104	1,073	1,052	230
	% Expected value	**102**	**95**	**104**	**87**	**99**
8	pg/mL	455	602	511	581	119
	% Expected value	**96**	**104**	**99**	**96**	**102**
16	pg/mL	229	284	230	286	60
	% Expected value	**96**	**98**	**89**	**95**	**102**

Dilution Factor	Interpolated value	Raji Extract 2. 5 µg/mL	HepG2 Extract 10 µg/mL	HeLa Extract 20 µg/mL
Undiluted	pg/mL	1,837	7,778	704
	% Expected value	**100**	**100**	**100**
2	pg/mL	925	4,286	357
	% Expected value	**101**	**110**	**101**
4	pg/mL	480	1,911	185
	% Expected value	**104**	**98**	**106**
8	pg/mL	239	842	91
	% Expected value	**104**	**87**	**104**
16	pg/mL	113	443	46
	% Expected value	**98**	**91**	**110**

PRECISION –

Mean coefficient of variations of interpolated values from 2 concentrations of ICAM-1 protein within the working range of the assay.

	Intra-Assay	Inter-Assay
n=	6	24
CV (%)	6	7

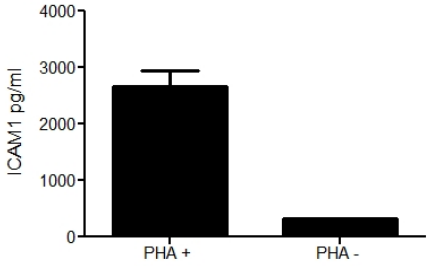


Figure 2. Specificity of ICAM-1 signal on stimulated and non stimulated media supernatants. Human PBMCs were cultured in RPMI supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin. Cells were cultured for 2 days at 37ºC in the presence or absence of PHA. The concentrations of ICAM-1 were interpolated from the calibration curve and corrected for sample dilution. The mean ICAM-1 concentration was determined to be 320 pg/mL in unstimulated PBMC supernatants and 2,654 pg/mL in stimulated PBMC supernatants

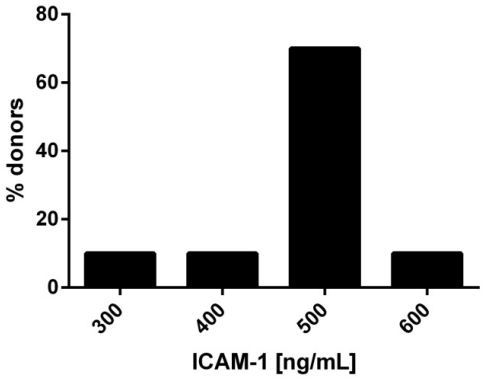


Figure 3. Frequency histogram of ICAM-1 levels in serum of individual normal healthy donors. The levels of ICAM-1 in serum samples were tested from ten individual healthy donors. Levels were interpolated from the standard curve and corrected for sample dilution. The levels of ICAM-1 are shown for the percentage of individuals within each 100 ng/mL bin center of the distribution. The mean level of ICAM-1 was 469 ng/mL with a range of 347 to 629 ng/mL and a standard deviation of 77 ng/mL.

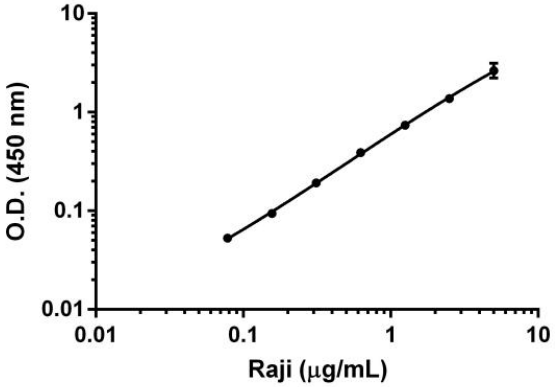


Figure 4. Example of ICAM1 dynamic range in Raji cell extracts. The curve was prepared by loading 5 µg/mL of Raji cell extracts, followed by a 1:2 titration series. Background-subtracted data values (mean +/- SD) are graphed.

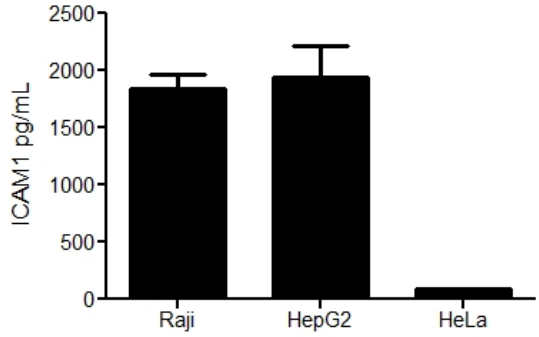


Figure 5. Comparison of ICAM-1 levels in three human cell culture lysates. The levels of ICAM-1 protein were assessed in three human cell line lysates loaded at 2.5 µg/mL of protein. The raw OD 450nm signal for each sample was interpolated from an ICAM1 standard curve.

17. ASSAY SPECIFICITY

This kit detects native and recombinant ICAM-1 in human cell culture supernatant, plasma, serum and cell extracts

18. SPECIES REACTIVITY

This kit recognizes human ICAM-1 protein.

Other species reactivity was determined by measuring 100X diluted serum samples of various species, interpolating the protein concentrations from the human standard curve, and expressing the interpolated concentrations as a percentage of the protein concentration in human serum assayed at the same dilution.

Reactivity < 3% was determined for the following species:

- Mouse
- Rat
- Hamster
- Guinea Pig
- Rabbit
- Dog
- Goat
- Pig
- Cow

Please contact our Scientific Support team for more information.

19. TROUBLESHOOTING

Problem	Cause	Solution
Difficulty pipetting lysate; viscous lysate.	Genomic DNA solubilized	Prepare 1X Cell Extraction Buffer PTR (without enhancer). Add enhancer to lysate after extraction.
Poor standard curve	Inaccurate Pipetting	Check pipettes
	Improper standard dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; increase to 2 or 3 hour standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
	Incubation times with TMB too brief	Ensure sufficient incubation time until blue color develops prior addition of Stop solution
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions.
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store your reconstituted standards at -80°C, all other assay components 4°C. Keep TMB Development Solution protected from light.
Precipitate in Diluent	Precipitation and/or coagulation of components within the Diluent.	Precipitate can be removed by gently warming the Diluent to 37ºC.

20. **NOTES**

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)