Version 3a Last updated 10 November 2022

ab229436
Human IL-10 CatchPoint® SimpleStep ELISA® Kit

For the quantitative measurement of IL-10 in human serum, plasma, and cell culture supernatant samples.

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

Table of Contents

1. Overview

IL-10 *in vitro* CatchPoint SimpleStep ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of IL-10 protein in human serum, plasma, and cell culture supernatant samples

The CatchPoint 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. CatchPoint HRP Development Solution containing the Stoplight Red Substrate is added. During incubation, the substrate is catalyzed by HRP generating a fluorescent product. Signal is generated proportionally to the amount of bound analyte and the intensity is measured in a fluorescence plater reader at 530/570/590 nm Excitation/Cutoff/Emission.

Interleukin 10 (IL-10) is a cytokine involved in immunoregulation through the promotion of Th2 response and inhibition of Th1 responses. It is produced by cells of hematopoietic origin, keratinocytes, placental cytotrophoblasts, and hepatic stellate cells.

2. Protocol Summary

Prepare all reagents, samples, and standards as instructed

Add 50 µL standard or sample to appropriate wells

Add 50 µL Antibody Cocktail to all wells

Incubate at room temperature for 1 hour

Aspirate and wash each well three times with 350 µL 1X Wash Buffer PT

Add 100 µL of prepared CatchPoint HRP Development Solution to each well and incubate for 10 minutes

Read fluorescence at Ex/Cutoff/Em 530/570/590 nm

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.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handle with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at +4°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. 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.
- All data, except Typical Standard Curve and Sensitivity were collected using the colorimetric version of this kit (ab185986).

6. Materials Supplied

Item	Quantity	Storage Condition
Human IL-10 Capture Antibody 10X	600 µL	+4°C
Human IL-10 Detector Antibody 10X	600 µL	+4°C
Human IL-10 Lyophilized Recombinant Protein	2 Vials	+4°C
Antibody Diluent CPI	6 mL	+4°C
Wash Buffer PT 10X	20 mL	+4°C
Cell Extraction Enhancer Solution 50X	1 mL	+4°C
Stoplight Red Substrate Buffer	12 mL	+4°C
100X Stoplight Red Substrate	120 µL	+4°C
500X Hydrogen Peroxide (H_2O_2, 3%)	50 µL	+4°C
Sample Diluent NS	50 mL	+4°C
Sample Diluent NBP	20 mL	+4°C
SimpleStep Pre-Coated Black 96-Well Microplate	96 Wells	+4°C
Plate Seal	1	+4°C

7. Materials Required, Not Supplied

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

- Fluorescence microplate reader Ex/Cutoff/Em 530/570/590 nm.
- Method for determining protein concentration (BCA assay recommended).
- Deionized water.
- Multi- and single-channel pipettes.
- Tubes for standard dilution.
- Plate shaker for all incubation steps.
- Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

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 Cell Extraction Enhancer Solution 50X may precipitate when stored at + 4°C. To dissolve, warm briefly at + 37°C and mix gently. The Cell Extraction Enhancer Solution 50X can be stored at room temperature to avoid precipitation.
- The incubation times provided in this protocol were optimized for fastest results with good signal. It is possible to increase the signal with longer incubation times, further optimization might be necessary.
- Keep in mind any RFU values shown are relative, NOT absolute. RFU from one plate reader are not comparable to another, especially if different make or model.
- To avoid high background always add samples or standards to the well before the addition of the antibody cocktail.
- 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.
- Sample Diluent BP may contain precipitate, this is normal. If precipitate is not dissolved by gentle mixing, the precipitate may be dissolved by gentle warming and mixing at 37°C for 10 minutes. If precipitate remains, gently spin down and avoid visible precipitates when pipetting.
- 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 Wash Buffer PT:

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

9.2 Antibody Cocktail:

Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent CPI. 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 CPI. Mix thoroughly and gently.

9.3 Sample Diluent 50BP + Enhancer:

Prepare Sample Diluent 50BP + Enhancer by thoroughly mixing Sample Diluent NBP, then diluting Sample Diluent NBP and 50X Cell Extraction Enhancer Solution with 1X Wash Buffer PT. To make 10 mL Sample Diluent 50BP + Enhancer, combine 4.8 mL 1X Wash Buffer PT, 5 mL Sample Diluent NBP, and 200 µL Cell Extraction Enhancer Solution 50X. Mix thoroughly by vortexing and heating gently in a warm water bath until debris is completely dissolved. If required, protease inhibitors can be added.

9.4 Sample Diluent 50BP (without enhancer):

Prepare Sample Diluent 50BP by thoroughly mixing Sample Diluent NBP, then diluting Sample Diluent NBP with 1X Wash Buffer PT. To make 10 mL Sample Diluent 50BP (without

enhancer), combine 5 mL 1X Wash Buffer PT and 5 mL Sample Diluent NBP. Mix thoroughly by vortexing and heating gently in a warm water bath until debris is completely dissolved. If required, protease inhibitors can be added.

9.5 Sample Diluent NS + Enhancer:

Prepare Sample Diluent NS + Enhancer by combining Sample Diluent NS and 50X Cell Extraction Enhancer Solution. To make 10 mL Sample Diluent NS + Enhancer, combine 9.8 mL Sample Diluent NS and 200 µL Cell Extraction Enhancer Solution 50X. Mix thoroughly and gently. If required, protease inhibitors can be added.

9.6 CatchPoint HRP Development Solution

Just prior to use prepare CatchPoint HRP Development Solution by diluting the 100X Stoplight Red Substrate and the 500X Hydrogen Peroxide in Stoplight Red Substrate Buffer.

For example, to make 6 mL of the CatchPoint HRP Development Solution combine 60 µL 100X Stoplight Red Substrate and 12 µL of 500X Hydrogen Peroxide with 5.928 mL Stoplight Red Substrate Buffer. Mix thoroughly and gently.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.
- 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 IL-10 by adding that volume of appropriate diluent (see below) indicated on the label. Alternatively, if the vial has a mass identified, reconstitute the IL-10 standard by adding 500 µL appropriate diluent. Hold at room temperature for 10 minutes and mix gently. This is the 11.6 ng/mL **Stock Standard** Solution.

For **cell culture supernatant samples,** reconstitute the IL-10 protein standard by adding Sample Diluent NS + Enhancer. For **serum and plasma samples,** reconstitute the IL-10 protein standard by adding Sample Diluent 50BP + Enhancer.

10.1.1 Label 14 tubes, Standards 1– 14.

10.1.2 Add 180 µL appropriate diluent (see step 10.1) into tube number 1 and 150 µL of appropriate diluent into numbers 2-14.

10.1.3 Use the Stock Standard to prepare the following dilution series. Standard #14 contains no protein and is the Blank control.

Standards will be added to the plate in step 13.3. If desired all 14 standards can be used for a full standard curve. Alternatively, to commit fewer wells to the standard curve, select a subset of at least 7 standards plus the blank control. If 7 standards are desired, we recommend standards #3-9.

Standard #	Dilution Sample	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	Stock	128	180	11,547	4,800
2	Standard#1	150	150	4,800	2400
3	Standard#2	150	150	2,400	1200
4	Standard#3	150	150	1,200	600
5	Standard#4	150	150	600	300
6	Standard#5	150	150	300	150
7	Standard#6	150	150	150	75
8	Standard#7	150	150	75.00	37.50
9	Standard#8	150	150	37.50	18.75
10	Standard#9	150	150	18.75	9.38
11	Standard#10	150	150	9.38	4.69
12	Standard#11	150	150	4.69	2.34
13	Standard#12	150	150	2.34	1.17
14	None	0	150	0	0

11. Sample Preparation

Typical Sample Dynamic Range	
Sample Type	**Range**
Serum*	≤ 50%
Plasma – Citrate*	≤ 50%
Plasma – Heparin*	≤ 25%
Plasma – EDTA*	≤ 25%
1.5% PHA-Stimulated PBMC cell culture supernatant	3.1 – 50%

***Based on spiked sample.**

11.1 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. Dilute samples at least 1:2 into Sample Diluent 50 BP (**without enhancer**), add 50X Cell Extraction Enhancer Solution to 1X, and assay. Any further dilutions should be made into Sample Diluent 50BP + Enhancer. Store un-diluted serum at -20°C or below. Avoid repeated freeze-thaw cycles.

11.2 Plasma:

Collect plasma using citrate, EDTA or heparin. Centrifuge samples at 2,000 x g for 10 minutes. Dilute samples at least 1:2 into Sample Diluent 50BP (**without enhancer**), add 50X Cell Extraction Enhancer Solution to 1X, and assay. Any further dilutions should be made into Sample Diluent BP + Enhancer. Store un-diluted plasma samples at -20°C or below for up to 3 months. 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. Collect supernatants and assay. Dilute samples as needed into Sample Diluent NS (**without enhancer**), add 50X Cell Extraction Enhancer Solution to 1X, and assay. Any further dilutions should be made into Sample Diluent NS + Enhancer. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

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 fluorescence or "edge effects" have not been observed with this assay.
- Ensure plate and all materials are equilibrated to room temperature during use.
- Cover the plate with a plate seal during incubation steps.

13. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.
- Prepare all reagents, working standards, and samples as directed in the previous sections.

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 4°C storage.

13.3 Add 50 µL of all sample or standard to appropriate wells.

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

13.5 Seal the 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. Wash Buffer PT should remain in wells for at least 10 seconds. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and tap gently against clean paper towels to remove excess liquid.

13.7 Add 100 µL of prepared CatchPoint HRP Development Solution to each well and incubate for 10 minutes in the dark on a plate shaker set to 400 rpm. Further optimization of incubation time vs signal strength can be performed if needed.

13.8 Record the fluorescence at Ex/Cutoff/Em 530/570/590 nm. If using a Molecular Devices' plate reader supported by SoftMax® Pro software, a preconfigured protocol for these CatchPoint SimpleStep ELISA Kits is available with all the protocol and analysis settings.

Mode:	Fluorescence
Instrument settings:	Endpoint
Excitation:	530 nm
Cutoff:	570 nm
Emission:	590 nm
Sensitivity:	6 flashes/read or 200ms
PMT:	Auto
Auto calibrate:	On
Read:	Top
Read Height:	1*

*For microplate readers with Pre-Read Optimization option, the Read Height as well as Microplate Optimization is recommended before the first read.

13.9 Analyze the data as described below.

14. Calculations

14.1 Preconfigured protocols are available when using SoftMax Pro software from Molecular Devices

14.2 Calculate the average fluorescence value for the blank control (zero) standards. Subtract the average blank control standard fluorescence value from all other fluorescence values.

14.3 Create a standard curve by plotting the average blank control subtracted fluorescence value for each standard concentration (y-axis) against the target protein concentration (x-axis) of the standard. Use graphing software to draw the best smooth curve through these points to construct the standard curve.

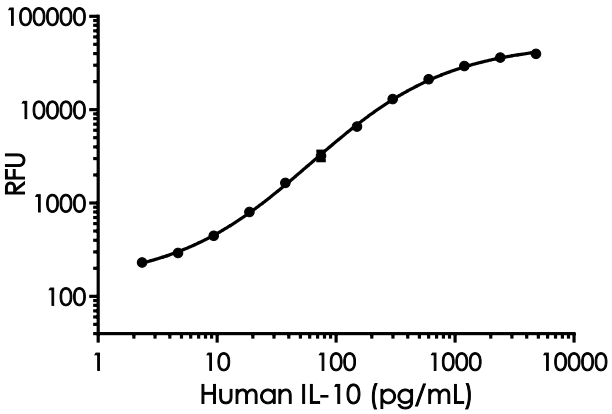
Δ Note: Most fluorescence reader software or graphing software will plot these values and fit a curve to the data. A four-parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g. linear, semi-log, log/log, 4-parameter logistic) can also be tested to determine if it provides a better curve fit to the standard values.

14.4 Determine the concentration of the target protein in the sample by interpolating the blank control subtracted fluorescence values against the standard curve. Multiply the resulting value by the appropriate sample dilution factor, if used, to obtain the concentration of target protein in the sample.

14.5 Samples generating fluorescence values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at fluorescence values less than that of the lowest standard should be retested in a less dilute form.

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			
Concentration (pg/mL)	RFU		Mean RFU
	1	2	
0	179	185	182
2.3	228	235	231
4.7	292	291	292
9.4	437	456	447
18.8	776	836	806
37.5	1,632	1,659	1,646
75	2,951	3,479	3,215
150	6,474	6,710	6,592
300	12,718	13,260	12,989
600	20,962	21,578	21,270
1,200	28,926	29,961	29,444
2,400	36,106	36,417	36,262
4,800	39,768	39,766	39,767

Figure 1. Example of human IL-10 standard curve in Sample Diluent NS + Enhancer. The IL-10 standard curve was prepared as described in Section 10. Raw data generated on SpectraMax M4 Multi-Mode Microplate Reader is shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

16. Calibration

This immunoassay is calibrated against a highly purified human IL-10. The NIBSC/WHO unclassified purified human IL-10 preparation 93/722 was evaluated in this kit.

The dose response curve of the unclassified standard IL-10 parallels the CatchPoint SimpleStep ELISA kit standard curve. To convert sample values obtained with the CatchPoint SimpleStep Human IL-10 ELISA kit to approximate NIBSC 93/722 units, use the equation below.

NIBSC (93/722) approximate value (IU/mL) = 0.01236 x CatchPoint SimpleStep Human IL-10 value (pg/mL).

17. Typical Sample Values

SENSITIVITY –

The calculated minimal detectable dose (MDD) is 2.1 pg/mL. The MDD was determined by calculating the mean of zero standard replicates (n=22) and adding 2 standard deviations then extrapolating the corresponding concentration.

RECOVERY –

Three concentrations of IL-10 recombinant standard protein were spiked in duplicate to the indicated biological matrix to evaluate signal recovery in the working range of the assay.

Sample Type	Average % Recovery	Range (%)
Serum (50%)	109	108 – 112
Plasma – Citrate (50%)	111	105 – 119
Plasma – Heparin (25%)	116	113 – 117
Plasma – EDTA (25%)	109	103 – 118
Stimulated PBMC Cell Culture Supernatant (25%)	108	105 - 111
Cell Culture Media (95%)*	106	102 - 111

*Media is RPMI 1640 containing 10% fetal calf serum.

Recombinant IL-10 was spiked into the following biological samples and diluted in a 2-fold dilution series in Sample Diluent 50BP + Enhancer.

Dilution Factor	Interpolated value	50% Human Serum	50% Human Plasma (Citrate)	25% Human Plasma (Heparin)	25% Human Plasma (EDTA)
Undiluted	pg/mL	2410	2293	2279	2110
	% Expected value	100	100	100	100
2	pg/mL	1078	1052	1079	1042
	% Expected value	89	92	95	99
4	pg/mL	526	527	531	523
	% Expected value	87	92	93	99
8	pg/mL	243	260	251	266
	% Expected value	81	91	88	101
16	pg/mL	118	113	126	123
	% Expected value	83	87	88	93

Native IL-10 was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS + Enhancer.

Dilution Factor	Interpolated value	50% Human 1.5% PHA-Stimulated PBMC SN
Undiluted	pg/mL	227
	% Expected value	100
2	pg/mL	119
	% Expected value	105
4	pg/mL	59
	% Expected value	103
8	pg/mL	29
	% Expected value	101
16	pg/mL	14
	% Expected value	101

50% pooled serum and plasma (EDTA, Heparin, Citrate) samples from healthy donors was measured in duplicate. All values were below the detectable range of the assay.

50% serum from ten individual healthy human male donors was measured in duplicate. All values were below the detectable range of the assay.

PRECISION –

Mean coefficient of variations of interpolated values of IL-10 from three concentrations of stimulated PBMC cell culture supernatant within the working range of the assay.

	Intra-Assay	Inter-Assay
n =	5	3
CV(%)	5.2	3.2

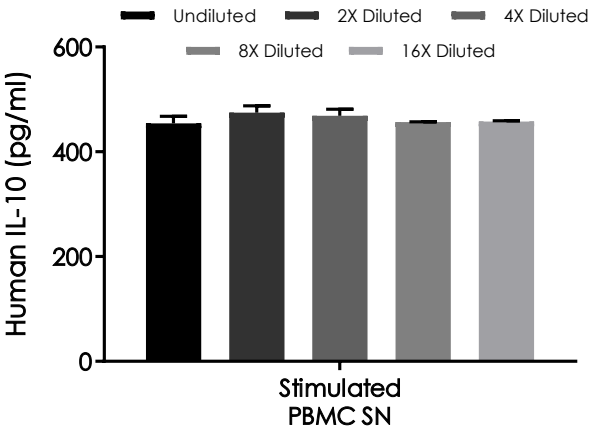


Figure 2. Interpolated concentrations of native IL-10 in human PBMC cell culture supernatant samples. The concentrations of IL-10 were measured in duplicates, interpolated from the IL-10 standard curves and corrected for sample dilution. Undiluted samples are as follows: stimulated PBMC cell culture supernatant 50%. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean IL-10 concentration was determined to be 462 pg/ml in stimulated PBMC cell culture supernatant. PBMC cell culture supernatant samples were stimulated for 46 hours with 1.5% PHA. Unstimulated PBMC cell culture supernatant samples were also measured; all values were below the detectable range of the assay.

18. Assay Specificity

This kit recognizes both native and recombinant human IL-10 protein in serum, plasma, and cell culture supernatant samples only.

Cell culture and tissue extract samples have not been tested with this kit.

CROSS REACTIVITY

Recombinant mouse and rat IL-10 were prepared at 50 ng/mL and 0.6 ng/mL each and assayed for cross reactivity. No cross-reactivity was observed.

INTERFERENCE

Recombinant human IL-10 R alpha and human IL-10 R beta were each prepared at 50 ng/mL and 0.6 ng/mL and tested for interference. No interference with was observed.

19. Species Reactivity

This kit recognizes human IL-10 protein.

Other species reactivity was determined by measuring 50% serum samples of various species, interpolating the IL-10 protein concentrations from the human standard curve, and expressing the interpolated concentrations as a percentage of the IL-10 protein concentration in human serum assayed at the same dilution.

Reactivity < 3% was determined for the following species:

- Mouse
- Rat
- Cow

Please contact our Technical Support team for more information.

20. Troubleshooting

Problem	Reason	Solution
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 CatchPoint HRP Development Solution too brief	Read plate again after longer incubation time
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 Stoplight Red Substrate 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 37°C.

21. 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)