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ab270891
Human CXCL9 CatchPoint® SimpleStep ELISA® Kit

For the quantitative measurement of CXCL9 in human cell culture supernatants and cell and tissue extract samples.

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

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1. Overview

CXCL9 *in vitro* CatchPoint SimpleStep ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of CXCL9 protein in human cell culture supernatants and cell and tissue extract 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.

CXCL9 is a small cytokine belonging to the CXC chemokine subfamily that lacks an ELR motif in front of the first cysteine. CXCL9, also known as MIG (Monokine Induced by Gamma Interferon) is a T-cell chemoattractant, which is induced by Interferon Gamma, This subfamily also includes Interferon Gamma Induced Protein 10 (IP-10 or CXCL10) and Interferon Inducible T-cell Alpha Chemoattractant (I-TAC or CXCL11) whose genes are located near the gene for CXCL9 on human chromosome 4, CXCL9, IP-10, and I-TAC all elicit their chemotactic functions by interacting with the G protein coupled chemokine receptor CXCR3 (GPR9 or CD183).

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 four 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 (ab219047).

6. Materials Supplied

Item	Quantity	Storage Condition
Human CXCL9 Capture Antibody 10X	600 µL	+4°C
Human CXCL9 Detector Antibody 10X	600 µL	+4°C
Human CXCL9 Lyophilized Recombinant Protein	2 Vials	+4°C
Antibody Diluent 4BI	6 mL	+4°C
Wash Buffer PT 10X	20 mL	+4°C
Cell Extraction Buffer PTR 5X	10 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
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.
- 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):

Prepare 1X Cell Extraction Buffer PTR by diluting Cell Extraction Buffer PTR 5X to 1X with deionized water. To make 10 mL 1X Cell Extraction Buffer PTR combine 8 mL deionized water and 2 mL Cell Extraction Buffer PTR 5X. Mix thoroughly and gently. If required protease inhibitors can be added.

9.2 1X Wash Buffer PT:

Prepare 1X Wash Buffer PT by diluting Wash Buffer PT 10X with deionized water. To make 100 mL 1X Wash Buffer PT combine 10 mL Wash Buffer PT 10X with 90 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.

9.4 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 CXCL9 protein standard by adding that volume of dilutant indicated on the label. Alternatively, if the vial has a mass identified, reconstitute the CXCL9 protein standard by adding 500 µL dilutant. Hold at room temperature for 10 minutes and mix gently. This is the 3000 pg/mL **Stock Standard** Solution.

10.2 For **cell culture supernatant samples measurements**, reconstitute the CXCL9 protein standard in Sample Diluent NS. For **cell and tissue extract samples measurements**, reconstitute the CXCL9 protein standard in of 1X Cell Extraction Buffer PTR.

10.3 Label 12 tubes, Standards 1– 12.

10.4 Add 300 µL of the **Stock Standard** into tube 1 and add 150 µL of appropriate diluent (see step 10.1) into numbers 2-14.

10.5 Use the Stock Standard to prepare the following dilution series. Standard #12 contains no protein and is the Blank control.

Standards will be added to the plate in step 13.3. If desired all 12 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 #1-7.

Standard #	Dilution Sample	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	Stock	300	0	3000	3000
2	Standard#1	150	150	3000	1500
3	Standard#2	150	150	1500	750
4	Standard#3	150	150	750	375
5	Standard#4	150	150	375	187.5
6	Standard#5	150	150	187.5	93.75
7	Standard#6	150	150	93.75	46.88
8	Standard#7	150	150	46.88	23.44
9	Standard#8	150	150	23.44	11.72
10	Standard#9	150	150	11.72	5.86
11	Standard#10	150	150	5.86	2.93
12	Standard#11	0	150	0.00	0.00

11. Sample Preparation

Typical Sample Dynamic Range	
Sample Type	Range
PHA-M Stimulated PBMC Cell Culture Supernatant (2 days)	0.16 – 2.5%
Unstimulated PBMC Cell Culture Supernatant (2 days)	6.25 – 100% (Neat)
PHA-M Stimulated PBMC Cell (2 days) Extract	12.5 – 200 µg/mL
Thyroid Tissue Extract	31.25 – 500 µg/mL

11.1 Cell Culture Supernatants:

Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants and assay or dilute samples into Sample Diluent NS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

11.2 Preparation of extracts from cell pellets:

11.2.1 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 4°C.

11.2.2 Rinse cells twice with PBS.

11.2.3 Solubilize pellet at $2x10^7$ cell/mL in chilled 1X Cell Extraction Buffer PTR.

11.2.4 Incubate on ice for 20 minutes.

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

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

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

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

11.3 Preparation of extracts from adherent cells by direct lysis (alternative protocol):

11.3.1 Remove growth media and rinse adherent cells 2 times in PBS.

11.3.2 Solubilize the cells by addition of chilled 1X Cell Extraction Buffer PTR directly to the plate (use 750 µL - 1.5 mL 1X Cell Extraction Buffer PTR per confluent 15 cm diameter plate).

11.3.3 Scrape the cells into a microfuge tube and incubate the lysate on ice for 15 minutes.

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

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

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

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

11.4 Preparation of extracts from tissue homogenates:

11.4.1 Tissue lysates are typically prepared by homogenization of tissue that is first minced and thoroughly rinsed in PBS to remove blood (dounce homogenizer recommended).

11.4.2 Homogenize 100 to 200 mg of wet tissue in 500 µL – 1 mL of chilled 1X Cell Extraction Buffer PTR. For lower amounts of tissue adjust volumes accordingly.

11.4.3 Incubate on ice for 20 minutes.

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

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

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

11.4.7 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 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 at www.softmaxpro.org.

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.

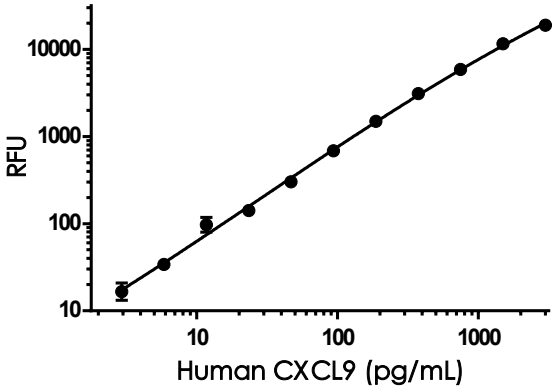
Δ Note: Most fluorescence reader software or graphing software will plot these values and fit a curve to the data. A four-parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g. linear, semi-log, log/log, 4-parameter logistic) can also be tested to determine if it provides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

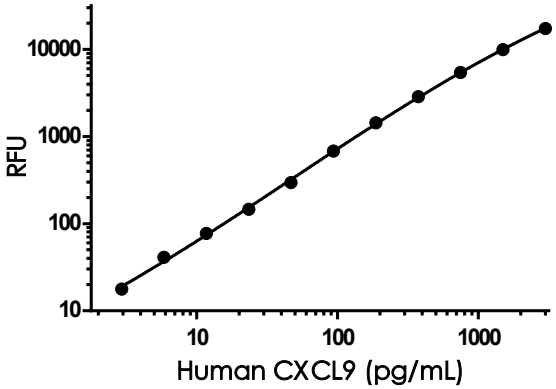
15. Typical Data

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	107.19	99.873	103.53
2.930	123.06	117.62	120.34
5.859	138.75	136.54	137.64
11.72	214.90	187.76	201.33
23.44	233.89	256.47	245.18
46.88	406.30	403.32	404.81
93.75	791.43	786.88	789.15
187.5	1,617.4	1,572.1	1,594.8
375.0	3,236.1	3,202.0	3,219.1
750.0	5,925.6	6,028.1	5,976.9
1,500	11,866	11,543	11,704
3,000	19,066	19,061	19,063

Figure 1. Example of human CXCL9 standard curve in Sample Diluent NS. The CXCL9 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.



Standard Curve Measurements			
Concentration (pg/mL)	RFU		Mean RFU
	1	2	
0	72.92	78.085	75.50
2.930	95.422	91.285	93.35
5.859	118.12	115.00	116.56
11.72	158.37	147.28	152.83
23.44	235.95	209.02	222.48
46.88	388.62	356.07	372.35
93.75	784.98	725.06	755.02
187.5	1,581.1	1,452.2	1,516.6
375.0	3,084.8	2,818.2	2,951.5
750.0	5,695.0	5,298.2	5,496.6
1,500	10,881	9,191.4	10,036
3,000	17,905	16,930	17,418

Figure 2. Example of human CXCL9 standard curve in 1X Cell Extraction Buffer PTR. The CXCL9 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. Typical Sample Values

SENSITIVITY –

The MDD was determined by calculating the mean of zero standard replicates and adding 2 standard deviations then extrapolating the corresponding concentration.

Sample Diluent Buffer	n=	Minimal Detectable Dose
Sample Diluent NS	22	2.6 pg/mL
1X Cell Extraction Buffer PTR	22	2.2 pg/mL

RECOVERY –

Three concentrations of CXCL9 recombinant 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 (%)
10% Unstimulated PBMC Cell	90	86 - 92
500 µg/mL Thyroid Tissue	109	104 - 116

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.

Native CXCL9 was measured in the following biological samples in a 2-fold dilution series. Cell culture supernatants (SN) sample dilutions are made in Sample Diluent NS. Cell and tissue extract sample dilutions are made in 1X Cell Extraction Buffer PTR.

Dilution Factor	Interpolated value	2.5% PHA-M Stimulated PBMC SN (2 days)	100% Unstimulated PBMC SN (2 days)	200 µg/mL PHA-M Stimulated PBMC Extract	500 µg/mL Thyroid Tissue Extract
Undiluted	pg/mL	572.2	1,491	1,065	718.5
	% Expected value	100	100	100	100
2	pg/mL	266.6	894.0	526.2	353.8
	% Expected value	93	120	99	98
4	pg/mL	123.1	403.5	258.4	214.3
	% Expected value	86	108	97	119
8	pg/mL	60.79	191.3	108.5	96.73
	% Expected value	85	103	81	108
16	pg/mL	29.46	103.3	58.05	51.56
	% Expected value	82	111	87	115

PRECISION –

Mean coefficient of variations of interpolated values of CXCL9 from three concentrations of PHA-M stimulated PBMC supernatant within the working range of the assay.

	Intra-Assay	Inter-Assay
n =	3	5
CV(%)	3.4	4.9

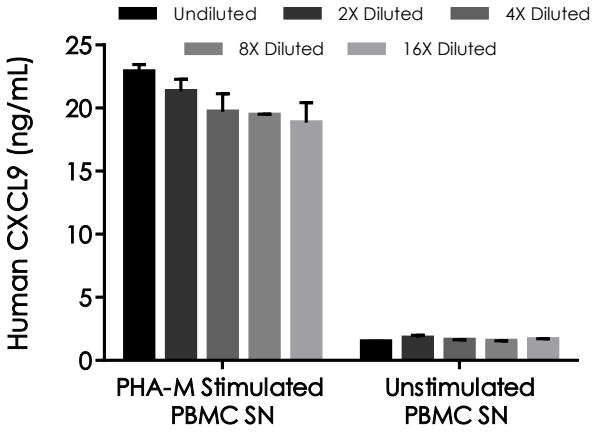


Figure 3. Interpolated concentrations of native CXCL9 in PHA-M stimulated and unstimulated human PBMC cell culture supernatant (2 days) samples. The concentrations of CXCL9 were measured in duplicates, interpolated from the CXCL9 standard curves and corrected for sample dilution. Undiluted samples are as follows: PHA-M stimulated PBMC supernatant 2.5% and unstimulated PBMC supernatant 100% (neat). The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean CXCL9 concentration was determined to be 20.4 ng/mL in neat PHA-M stimulated PBMC supernatant and 1.62 ng/mL in neat unstimulated PBMC supernatant.

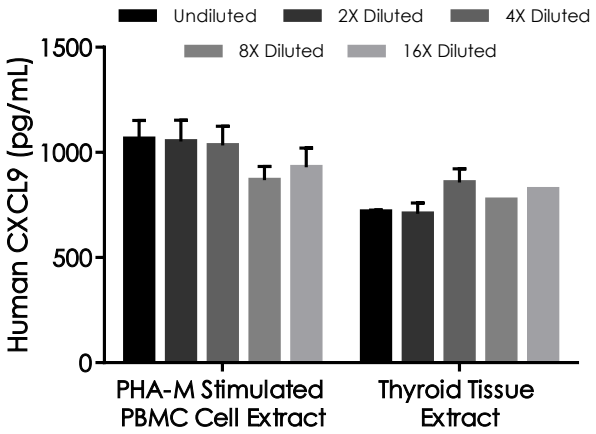


Figure 4. Interpolated concentrations of native CXCL9 in PHA-M stimulated human PBMC cell extract based on a 200 µg/mL extract load and human thyroid tissue extract based on a 500 µg/mL extract load. The concentrations of CXCL9 were measured in duplicate and interpolated from the CXCL9 standard curve and corrected for sample dilution. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean CXCL9 concentration was determined to be 993.9 pg/mL in PHA-M stimulated PBMC cell extract and 824.9 pg/mL in thyroid tissue extract.

17. Assay Specificity

This kit recognizes both native and recombinant human CXCL9 protein in cell culture supernatant and cell and tissue extract samples only.

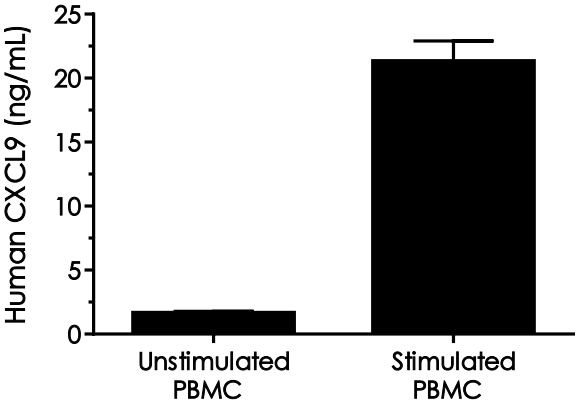


Figure 5. Comparison of CXCL9 in unstimulated and PHA-M stimulated human PBMC cell supernatants. Human PBMC cells were cultured in the absence or presence of 1.5% PHA-M for 2 days. The concentrations of CXCL9 were measured in three different dilutions of the supernatant samples in duplicates and interpolated from the CXCL9 standard curve. The interpolated values are plotted (mean +/- SD, n=3). The mean CXCL9 concentration was determined to be 21.3 ng/mL in PHA-M stimulated PBMC cell supernatant, 1.6 ng/mL in unstimulated supernatants and undetectable in media (not shown).

Saliva and milk samples have not been tested with this kit.

Urine, bronchial lavage, serum, and plasma samples are incompatible with this kit.

CROSS REACTIVITY

Recombinant human GRO-alpha (CXCL1), GRO-beta (CXCL2) and GRO-gamma (CXCL3) were prepared at 50 ng/mL and 1 ng/mL and assayed for cross reactivity. No cross-reactivity was observed.

18. Species Reactivity

This kit recognizes human CXCL9 protein.

Other species reactivity was determined by measuring mouse recombinant protein prepared within the working range of the assay. No mouse recombinant protein reactivity was observed.

Please contact our Technical Support team for more information.

19. Troubleshooting

Problem	Reason	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 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.

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)