Quantikine® ELISA

Human XCL1/Lymphotactin Immunoassay

Catalog Number DXCL10

For the quantitative determination of human Lymphotactin concentrations in cell culture supernates, serum, and plasma.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Lymphotactin, also known as XCL1, ATAC and SCM-1, is a 10 kDa glycosylated protein in the C chemokine family (1-5). XCL2 is a closely related chemokine that differs from Lymphotactin by only two amino acids (aa) (6). Human Lymphotactin shares 61% aa sequence identity with mouse Lymphotactin. XCL2 has no rodent ortholog. Unlike most chemokines, which contain two characteristic disulfide bonds, C chemokines have only a single disulfide bond. Lymphotactin is secreted by activated NK cells, Th1 cells, and $CD8^+$ T cells as part of a Th1 response upon infection (7-9). Lymphotactin is also expressed by effector memory $CD8^+$ T cells, mast cells, and medullary thymic epithelial cells (10-12).

Lymphotactin binds and activates a G protein-coupled receptor, XCR1 (13, 14). XCR1 is expressed on a subset of antigen cross-presenting dendritic cells (5, 15). Binding of Lymphotactin to XCR1 initiates dendritic cell chemotaxis as well as dendritic cell mediated differentiation of cytotoxic T cells and regulatory T cells (Tregs) (10, 16). Dysregulated expression of Lymphotactin in Tregs can contribute to allergic asthma responses (17). XCR1 is also expressed on ovarian carcinoma cells and may contribute to cancer cell migration and proliferation in response to Lymphotactin or XCL2 (18).

Lymphotactin has been detected in the synovial fluid of rheumatoid arthritis patients and in the serum of patients with systemic sclerosis (19-21). It has also been identified in the aqueous humor in a rodent model of autoimmune anterior uveitis (22).

The Quantikine Human XCL1/Lymphotactin Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human Lymphotactin in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant human Lymphotactin and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human Lymphotactin showed linear curves that were parallel to the standard curves obtained using the Quantikine kit standards. These results indicate that this kit can be used to determine relative mass values for naturally occurring human Lymphotactin.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human Lymphotactin has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Lymphotactin present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human Lymphotactin is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of Lymphotactin bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples with the appropriate Calibrator Diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human Lymphotactin Microplate	894675	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human Lymphotactin.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Human Lymphotactin Conjugate	894676	21 mL of a polyclonal antibody specific for human Lymphotactin conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human Lymphotactin Standard	894677	Recombinant human Lymphotactin in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Assay Diluent RD1-88	895880	12 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD6-15	895244	21 mL of a buffered protein base with preservatives. *Used undiluted for serum/plasma samples. Used diluted 1:8 for cell culture supernate samples.*	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	6 mL of 2 N sulfuric acid.	
Plate Sealers	N/A	4 adhesive strips.	

* Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 50 mL and 500 mL graduated cylinders.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- Test tubes for dilution of standards and samples.
- Human Lymphotactin Controls (optional; available from R&D Systems).

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain ProClin® which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Please refer to the MSDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated for use in this assay.*
Grossly icteric or hemolyzed samples are not recommended for use in this assay.
Samples with abnormally high levels of Albumin interfere in this assay.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

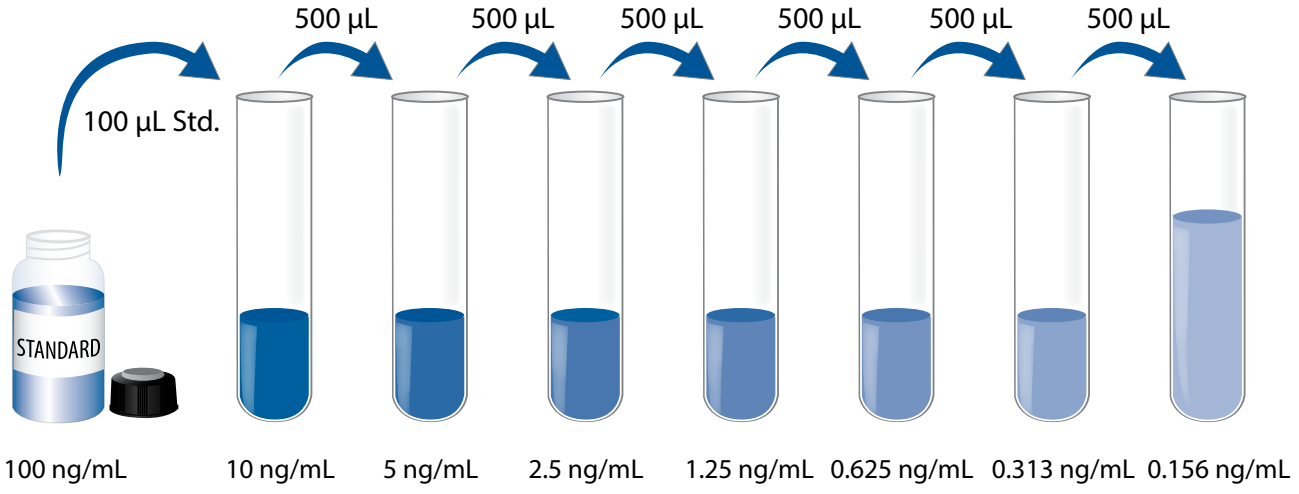
Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

Calibrator Diluent RD6-15 (diluted 1:8) - **For cell culture supernate samples only.** Add 3 mL of Calibrator Diluent RD6-15 to 21 mL of deionized or distilled water to prepare 24 mL of Calibrator Diluent RD6-15 (diluted 1:8).

Human Lymphotactin Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human Lymphotactin Standard with deionized or distilled water. This reconstitution produces a stock solution of 100 ng/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Note: *Diluted standard must be used within 30 minutes.*

Pipette 900 µL of Calibrator Diluent RD6-15 (diluted 1:8) (*for cell culture supernate samples*) or Calibrator Diluent RD6-15 (*for serum/plasma samples*) into the 10 ng/mL tube. Pipette 500 µL of the appropriate Calibrator Diluent into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 10 ng/mL standard serves as the high standard. The appropriate Calibrator Diluent serves as the zero standard (0 ng/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples, controls, and standards be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 µL of Assay Diluent RD1-88 to each well.
4. Add 100 µL of Standard, control, or sample per well. Cover with the adhesive strip provided. Incubate for 3 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 200 µL of Human Lymphotactin Conjugate to each well. Cover with a new adhesive strip. Incubate for 1 hour at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
9. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

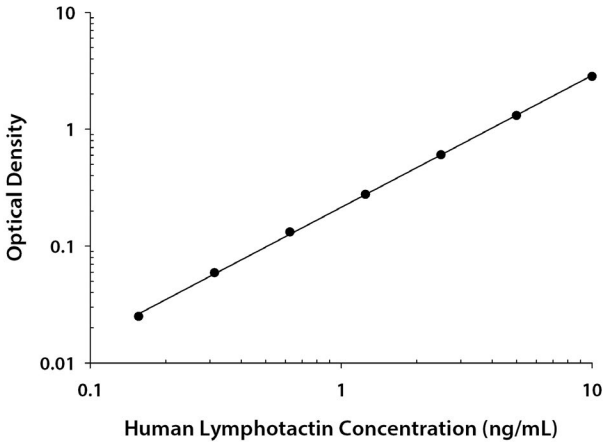
Create a standard curve by reducing the data using computer software capable of generating a log/log curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on a log/log graph. The data may be linearized by plotting the log of the human Lymphotactin concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

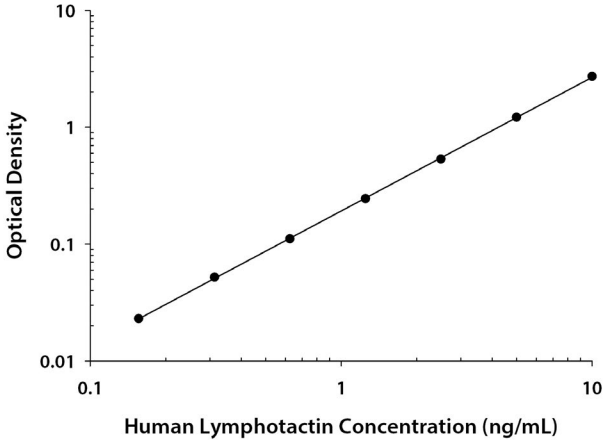
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE ASSAY



(ng/mL)	O.D.	Average	Corrected
0	0.009 0.009	0.009	—
0.156	0.033 0.034	0.034	0.025
0.313	0.067 0.069	0.068	0.059
0.625	0.138 0.144	0.141	0.132
1.25	0.284 0.288	0.286	0.277
2.5	0.601 0.627	0.614	0.605
5	1.288 1.352	1.320	1.311
10	2.824 2.859	2.842	2.833

SERUM/PLASMA ASSAY



(ng/mL)	O.D.	Average	Corrected
0	0.013 0.016	0.015	—
0.156	0.037 0.038	0.038	0.023
0.313	0.066 0.068	0.067	0.052
0.625	0.124 0.127	0.126	0.111
1.25	0.256 0.264	0.260	0.245
2.5	0.545 0.552	0.549	0.534
5	1.229 1.233	1.231	1.216
10	2.727 2.744	2.736	2.721

PRECISION

Intra-assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

CELL CULTURE SUPERNATE ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (ng/mL)	0.920	2.83	5.57	1.01	3.08	6.29
Standard deviation	0.024	0.078	0.116	0.095	0.278	0.494
CV (%)	2.6	2.8	2.1	9.4	9.0	7.9

SERUM/PLASMA ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (ng/mL)	1.12	3.27	6.66	1.14	3.32	6.69
Standard deviation	0.031	0.085	0.184	0.084	0.199	0.374
CV (%)	2.8	2.6	2.8	7.4	6.0	5.6

RECOVERY

The recovery of human Lymphotactin spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	109	99-116%
Serum (n=4)	98	89-107%
EDTA plasma (n=4)	95	86-100%
Heparin plasma (n=4)	93	87-101%

SENSITIVITY

Forty-eight assays were evaluated and the minimum detectable dose (MDD) of human Lymphotactin ranged from 0.005-0.023 ng/mL. The mean MDD was 0.012 ng/mL.

The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of human Lymphotactin were diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=4)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)
1:2	Average % of Expected	98	94	94	92
	Range (%)	94-103	91-95	89-98	91-96
1:4	Average % of Expected	97	94	94	91
	Range (%)	92-101	90-96	90-99	90-95
1:8	Average % of Expected	94	94	95	93
	Range (%)	91-98	90-98	89-99	89-97
1:16	Average % of Expected	90	92	93	92
	Range (%)	87-97	87-95	86-102	85-99

CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant human Lymphotactin manufactured at R&D Systems.

SAMPLE VALUES

Serum/Plasma - Thirty-five samples from apparently healthy volunteers were evaluated for the presence of human Lymphotactin in this assay. No detectable levels were observed. No medical histories were available for the donors used in this study.

Cell Culture Supernates:

HuT 78 human cutaneous T cell lymphoma cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, and 50 µM β-mercapthoethanol. An aliquot of the cell culture supernate was removed, assayed for human Lymphotactin, and measured 0.315 ng/mL.

$CD8^+$ T cells were isolated from human peripheral blood mononuclear cells. Cells were cultured unstimulated or stimulated with anti-human CD3 and anti-human CD28 antibodies for 6 days. On day 7, 10 ng/mL PMA and 500 ng/mL Ionomycin were added and cells were cultured for an additional 24 hours. Aliquots of the cell culture supernates were removed and assayed for human Lymphotactin.

Unstimulated (ng/mL)	Stimulated (ng/mL)
ND	2.69

ND=Non-detectable

SPECIFICITY

This assay recognizes natural and recombinant human Lymphotactin. This assay also recognizes human XCL2.

The factors listed below were prepared at 100 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 100 ng/mL in a mid-range recombinant human Lymphotactin control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

CCL1/I-309
CCL2/MCP-1
CCL3/MIP-1α
CCL4/MIP-1β
CCL5/RANTES
CCL7/MCP-3
CCL8/MCP-2
CCL11/Eotaxin
CCL13/MCP-4
CCL14a/HCC-1
CCL15/MIP-1δ/LKN-1
CCL16/HCC-4
CCL17/TARC
CCL18/PARC
CCL19/MIP-3β
CCL20/MIP-3α
CCL21/6Ckine
CCL22/MDC
CCL23/MPIF-1
CCL24/Eotaxin-2/MPIF-2
CCL25/TECK
CCL26/Eotaxin-3
CCL27/CTACK
CXCL1/GROα
CXCL2/GROβ
CXCL3/GROγ
CXCL5/ENA-78
CXCL6/GCP-2
CXCL7/NAP-2
CXCL8/IL-8
CXCL9/MIG
CXCL10/IP-10
CXCL11/I-TAC
CXCL12/SDF-1α
CXCL12/SDF-1β
CXCL13/BLC/BCA-1
XCR1

Recombinant mouse:

CCL1/TCA-3
CCL2/JE/MCP-1
CCL3/MIP-1α
CCL4/MIP-1β
CCL5/RANTES
CCL7/MARC
CCL9/10/MIP-1γ
CCL11/Eotaxin
CCL12/MCP-5
CCL17/TARC
CCL19/MIP-3β
CCL20/MIP-3α
CCL21/6Ckine
CCL22/MDC
CCL25/TECK
CCL27/CTACK
CXCL1/KC
CXCL9/MIG
CXCL10/IP-10/CRG-2
CXCL12/SDF-1α
CXCL13/BLC/BCA-1
XCL1/Lymphotactin

Other recombinants:

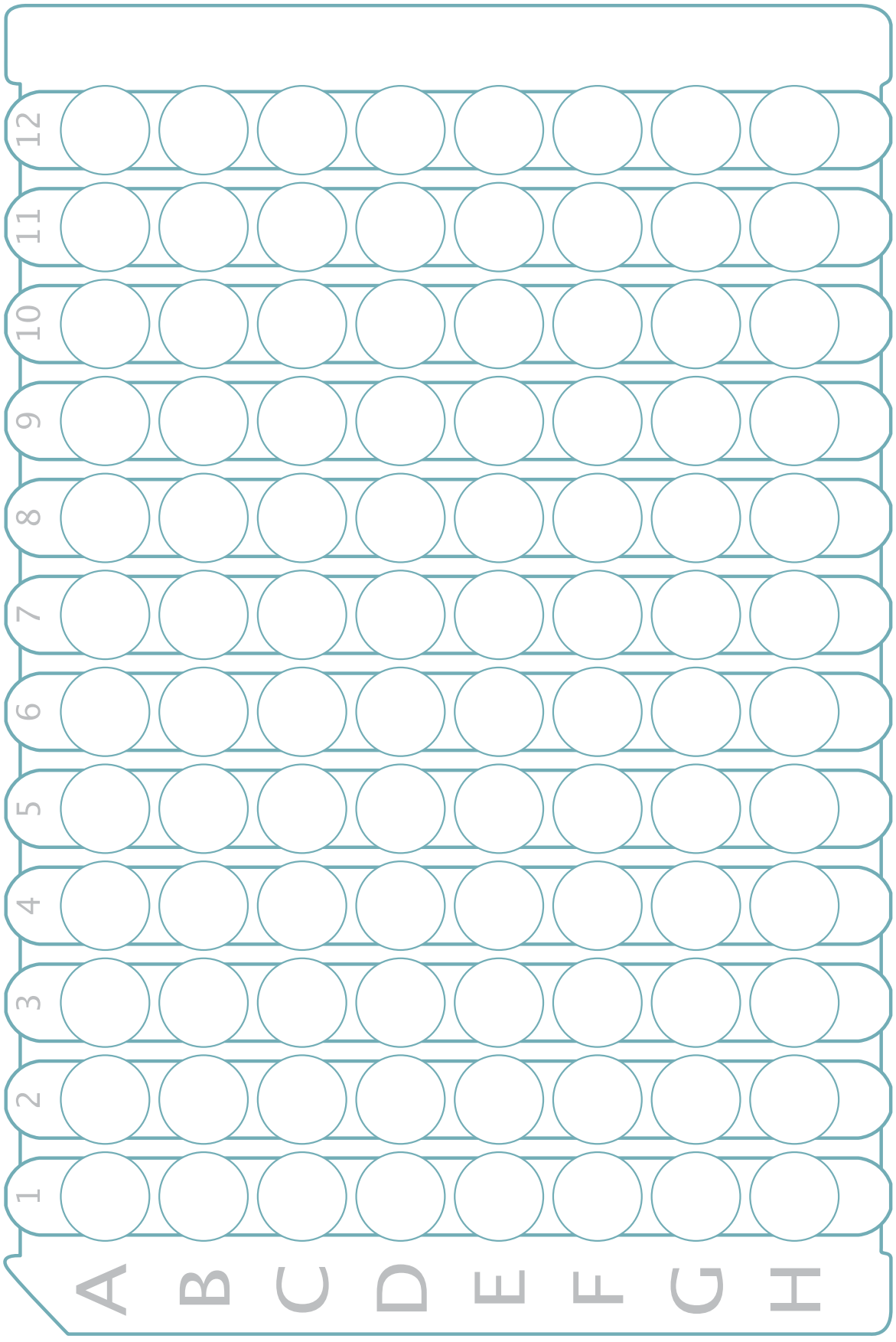
viral HHV-8/MIP-1β
viral MIP-3

REFERENCES

1. Kelner, G.S. *et al.* (1994) Science **266**:1395.
2. Muller, S. *et al.* (1995) Eur. J. Immunol. **25**:1744.
3. Yoshida, T. *et al.* (1995) FEBS Lett. **360**:155.
4. Dorner, B. *et al.* (1997) J. Biol. Chem. **272**:8817.
5. Kroczek, R.A. and V. Henn (2012) Front. Immunol. **3**:14.
6. Yoshida, T. *et al.* (1996) FEBS Lett. **395**:82.
7. Dorner, B.G. *et al.* (2002) Proc. Natl. Acad. Sci. USA **99**:6181.
8. Dorner, B.G. *et al.* (2004) J. Immunol. **172**:3119.
9. Ordway, D. *et al.* (2007) J. Leukoc. Biol. **82**:1221.
10. Lei, Y. *et al.* (2011) J. Exp. Med. **208**:383.
11. Feuser, K. *et al.* (2012) Cytokine **58**:178.
12. Jubin, V. *et al.* (2012) Immunol. Res. **52**:284.
13. Yoshida, T. *et al.* (1998) J. Biol. Chem. **273**:16551.
14. Shan, L. *et al.* (2000) Biochem. Biophys. Res. Commun. **268**:938.
15. Bachem, A. *et al.* (2012) Front. Immunol. **3**:214.
16. Dorner, B.G. *et al.* (2009) Immunity **31**:823.
17. Nguyen, K.D. *et al.* (2008) J. Immunol. **181**:5386.
18. Kim, M. *et al.* (2012) Mol. Cancer Res. **10**:1419.
19. Blaschke, S. *et al.* (2003) Arthritis Rheum. **48**:1858.
20. Endres, M. *et al.* (2010) Osteoarthritis Cartilage **18**:1458.
21. Muroi, E. *et al.* (2008) J. Rheumatol. **35**:834.
22. Yeh, P.T. *et al.* (2010) Graefes Arch. Clin. Exp. Ophthalmol. **248**:1737.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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