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R&D SYSTEMS

Quantikine® ELISA

Human CD23/Fcε RII Immunoassay

Catalog Number DCD230

For the quantitative determination of human CD23 concentrations in cell culture supernates, serum, plasma, and urine.

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

CD23, also called Fcε RII, is a member of the C-type lectin superfamily that is the low-affinity Fc receptor for IgE, the antibody isotype that mediates allergic inflammation (1-5). It is a 47 kDa type II transmembrane glycoprotein containing a short N-terminal cytoplasmic tail, a transmembrane domain, an extracellular stalk region that facilitates CD23 trimerization and enhances affinity for IgE, and a C-terminal C-type (Ca^{2+}-dependent) lectin domain (3-12). Two splice isoforms that differ only in their cytoplasmic domains are termed a and b (13-17). CD23a is constitutively expressed by B cells and intestinal epithelial cells (IEC). CD23b is induced on a variety of cell types including B cells, monocytes, eosinophils, IEC and airway smooth muscle cells by a range of exogenous stimuli, including IL-4 and GM-CSF. Soluble CD23 fragments (sCD23) of sizes ranging from 16 to 37 kDa are released from the cell surface and further processed via cleavage by metalloproteinases (principally ADAM10) and cysteine proteases (11, 12, 18-23). All fragments contain the C-terminal lectin domain, and the 25 kDa sCD23 fragment predominates in serum.

Membrane and soluble forms of CD23 each bind multiple ligands and exert diverse physiologic functions. For membrane CD23, intracellular trafficking regulates many of its functions. On IEC, membrane CD23-mediated transepithelial transport of IgE/allergen complexes to the underlying mast cells is thought to contribute to food allergies (24-26). B cell CD23a, when complexed with *HLA-DR* and antigen-IgE, undergoes efficient endocytosis to enhance the IgE-dependent antigen presentation to T cells (16). When expressed on the surface of macrophages, CD23b mediates phagocytosis of IgE-opsonized particles (13). CD23 plays a pivotal role in IgE homeostasis. Whereas binding of circulating IgE to membrane CD23 down-regulates further IgE production, binding of most forms of soluble CD23 to CD21 upregulates the level of IgE production by B cells (20, 27-30). Soluble CD23 also has cytokine-like activities. Soluble CD23 also binds α_m/β_2 (CD11b/CD18, MAC-1), α_x/β_2 (CD11c/CD18) and α_v integrins on monocytic cells to activate production of inflammatory cytokines (31-33).

In a number of pathological conditions, including allergy, rheumatoid arthritis and B cell chronic lymphocytic leukemia (B-CLL), CD23 is over-expressed and elevated levels of sCD23 are shed. In B-CLL, the increased expression of CD23 is regulated at least in part via Notch-2 signaling (34). Elevated plasma sCD23 levels correlate with the total tumor burden (35). In allergic patients, expression of CD23 is increased on monocytes, lung alveolar macrophages and circulating B cells (17, 36). In rheumatoid arthritis, synovial fluid sCD23 correlates with the presence of joint erosion (37).

The Quantikine® Human CD23/Fcε RII Immunoassay is a 4.5 hour solid phase ELISA designed to measure human CD23 in cell culture supernates, serum, plasma, and urine. It contains NS0-expressed recombinant human CD23 (extracellular domain) and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human CD23 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 human CD23.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human CD23 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any CD23 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human CD23 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 CD23 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, further dilute the samples with calibrator diluent and repeat the assay.
- Any variation in 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 CD23 Microplate	893561	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human CD23.	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 CD23 Conjugate	893562	12 mL of a polyclonal antibody specific for human CD23 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human CD23 Standard	893563	Recombinant human CD23 in a buffered protein solution with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Assay Diluent RD1-36	895272	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-3	895436	21 mL of a buffered protein base with preservatives.	
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	895174	23 mL of a diluted hydrochloric acid solution.	
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.
- 500 mL graduated cylinder.
- Test tubes for dilution of standards and samples.
- Human CD23 Controls (optional; R&D Systems®, Catalog # QC24).

PRECAUTIONS

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

Some components in this kit contain a preservative 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. Refer to the SDS 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.*

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, and assay immediately or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Serum, plasma, and urine samples require a 5-fold dilution. A suggested 5-fold dilution is 30 µL of sample + 120 µL of Calibrator Diluent RD5-3.

REAGENT PREPARATION

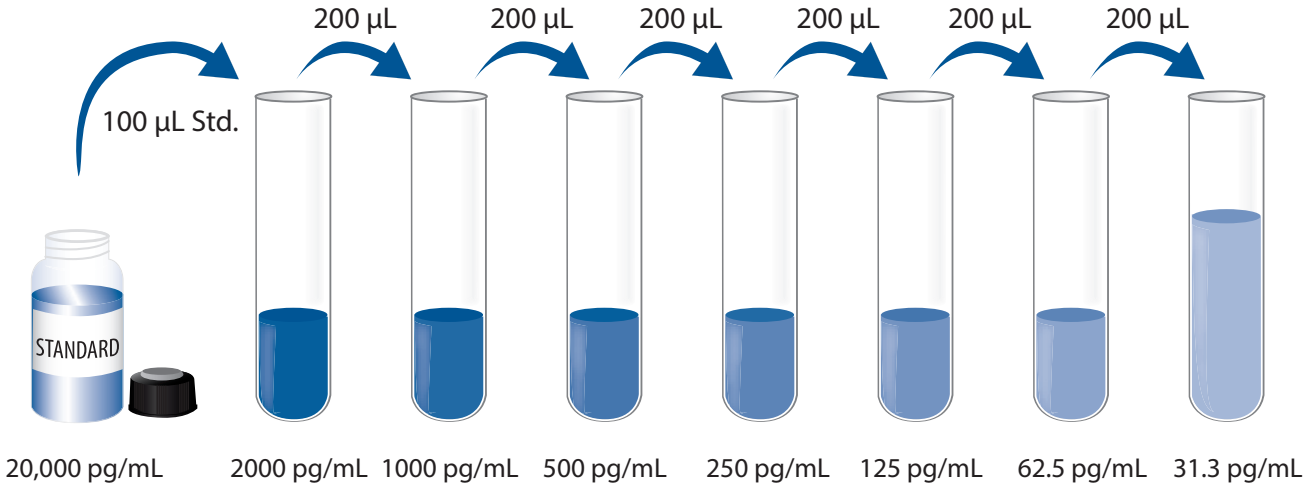
Bring all reagents to room temperature before use.

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 480 mL of 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. 100 µL of the resultant mixture is required per well.

Human CD23 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human CD23 Standard with deionized or distilled water. This reconstitution produces a stock solution of 20,000 pg/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.

Pipette 900 µL of Calibrator Diluent RD5-3 into the 2000 pg/mL tube. Pipette 200 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 2000 pg/mL standard serves as the high standard. Calibrator Diluent RD5-3 serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, samples, and controls 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 50 µL of Assay Diluent RD1-36 to each well.
4. Add 50 µL of standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 3 hours at room temperature. 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 100 µL of Human CD23 Conjugate to each well. Cover with a new adhesive strip. Incubate for 1 hour at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
9. Add 100 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.

*Samples may require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

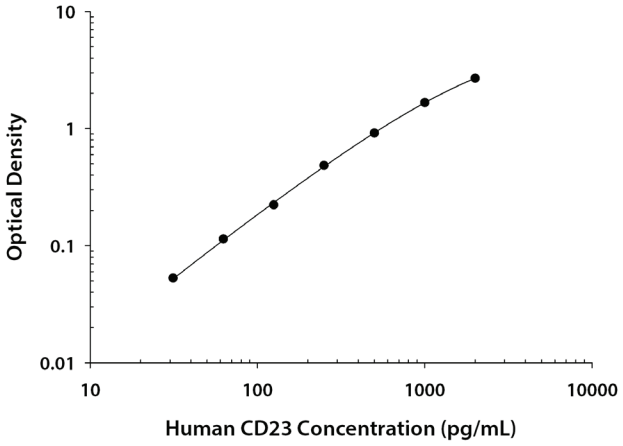
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) 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 the graph. The data may be linearized by plotting the log of the human CD23 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

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

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D.	Average	Corrected
0	0.047 0.047	0.047	—
31.3	0.099 0.100	0.100	0.053
62.5	0.155 0.167	0.161	0.114
125	0.266 0.274	0.270	0.223
250	0.494 0.568	0.531	0.484
500	0.893 1.030	0.962	0.915
1000	1.599 1.820	1.710	1.663
2000	2.626 2.847	2.737	2.690

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 forty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	40	40	40
Mean (pg/mL)	158	283	576	151	274	533
Standard deviation	13.5	17.8	40.8	13.7	22.4	40.2
CV (%)	8.5	6.3	7.1	9.1	8.2	7.5

RECOVERY

The recovery of human CD23 spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	105	94-114%
Serum (n=4)	94	85-108%
EDTA plasma (n=4)	99	89-105%
Heparin plasma (n=4)	98	90-112%
Urine (n=4)	100	85-113%

LINEARITY

To assess the linearity of the assay, samples containing high concentrations of human CD23 were diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture samples (n=4)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)	Urine (n=4)
1:2	Average % of Expected	101	102	104	104	101
	Range (%)	97-106	95-108	98-108	100-109	98-103
1:4	Average % of Expected	100	103	107	108	111
	Range (%)	98-104	97-115	101-112	104-111	109-114
1:8	Average % of Expected	99	101	106	109	109
	Range (%)	92-106	96-111	103-110	105-111	104-113
1:16	Average % of Expected	100	100	101	108	107
	Range (%)	97-105	88-109	90-110	96-113	99-114

SENSITIVITY

Forty assays were evaluated and the minimum detectable dose (MDD) of human CD23 ranged from 1.24-5.10 pg/mL. The mean MDD was 3.18 pg/mL.

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

CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant human CD23 (extracellular domain) produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma/Urine - Samples from apparently healthy volunteers were evaluated for the presence of human CD23 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=35)	2515	1235-5025	1004
EDTA plasma (n=35)	2732	1285-5355	1108
Heparin plasma (n=35)	2319	1115-5060	967
Urine (n=10)	3458	1057-9448	2503

Cell Culture Supernates:
Human peripheral blood lymphocytes (PBLs;1 x 10^6 cells/mL) were cultured in RPMI supplemented with 10% fetal bovine serum, 5 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10 µg/mL PHA for 6 days. Aliquots of the cell culture supernate were removed and assayed for levels of human CD23.

Condition	Day 6 (pg/mL)
Unstimulated	85.3
Stimulated	512

Human peripheral blood mononuclear cells (PBMCs; 1 x 10^6 cells/mL) were cultured in RPMI supplemented with 10% fetal bovine serum, 2 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10 ng/mL of recombinant human IL-2 for 24 hours. Aliquots of the cell culture supernates were removed and assayed for levels of human CD23.

Condition	Day 1 (pg/mL)
Unstimulated	114
Stimulated	117

SAMPLE VALUES *CONTINUED*

U937 human histiocytic lymphoma cells were cultured in RPMI supplemented with 10% fetal bovine serum and 2 mM L-glutamine. Cells were cultured unstimulated for 1 and 3 days. Aliquots of the cell culture supernates were removed, assayed for human CD23, and measured 411 pg/mL and 287 pg/mL, respectively.

N1186 human T cells were cultured in RPMI supplemented with 10% fetal bovine serum, 2 mM L-glutamine, and 15 ng/mL of recombinant human IL-2 for 10 days. An aliquot of the cell culture supernate was removed, assayed for human CD23, and measured 12,120 pg/mL.

Dendritic cells were cultured in RPMI supplemented with 10% fetal bovine serum, 5 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were cultured with 1000 U/mL of recombinant human GM-CSF and 500 U/mL of recombinant human IL-4 for 7 days. An aliquot of the cell culture supernate was removed, assayed for human CD23, and measured 12,864 pg/mL.

Raji human Burkitt's lymphoma cells were cultured in RPMI supplemented with 10% fetal bovine serum and 2 mM L-glutamine for 4 days. An aliquot of the cell culture supernate was removed, assayed for human CD23, and measured 2348 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant human CD23, including the 25 kDa fragment (aa 150-321) and the full length extracellular domain (aa 48-321).

The factors listed below were prepared at 50 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range recombinant human CD23 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

Ang-1
Ang-2
Integrin αL/CD11a
Integrin αM/CD11b
Integrin αX/CD11c
Fcγ RIII/CD16
Fcγ RIIIB/CD16b)
Siglec-2/CD22
Fcγ RIIA/CD32
Fcγ RIIA/CD32a
Fcγ RIIB/CD32b
Fcγ RIA/CD64a
CD40
CD40 Ligand
Epo
Fas
GM-CSF
IgG1 Fc region
IL-1ra
IL-2
IL-5
IL-6
IL-8
IL-10
IL-10 R
IL-11
IL-12 p70
IL-12/IL-23 p40
IL-14
IL-17
IL-20
IL-20 Rα
IL-22BP
IL-22 R
IL-24
IL-28A
SCF
TNF-α
TNF-β

Recombinant mouse:

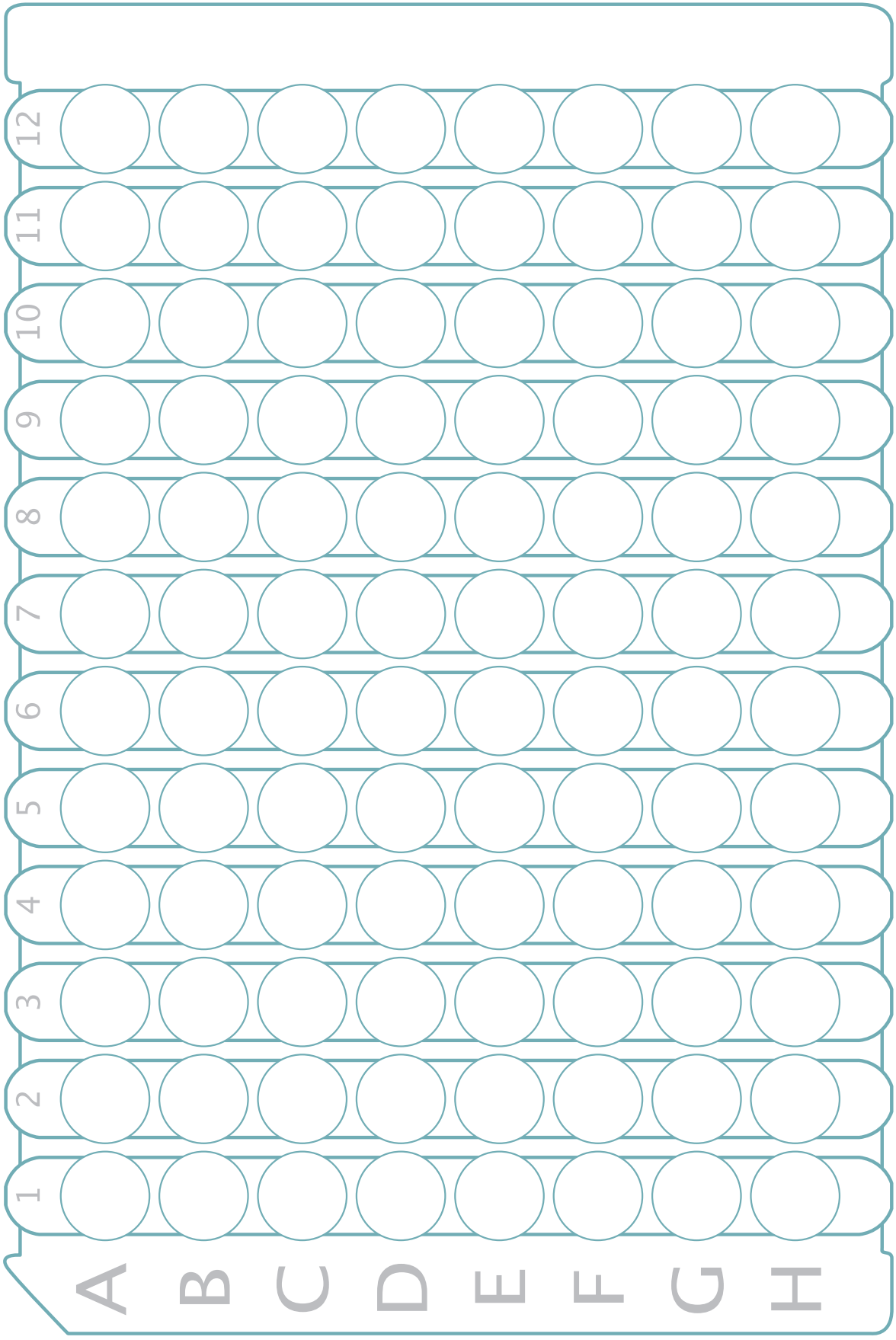
CD16-2
Siglec-2/CD22
Fcγ RIA/CD64

REFERENCES

1. Kijimoto-Ochiai, S. (2002) Cell. Mol. Life Sci. **59**:648.
2. Bruhns, P. *et al.* (2005) Curr. Opin. Immunol. **17**:662.
3. Bajorath, J. and A. Aruffo (1996) Protein Sci. **5**:240.
4. Drickamer, K. (1993) Curr. Opin. Struct. Biol. **3**:393.
5. Wurzburg, B.A. *et al.* (2006) Structure **14**:1049.
6. Ludin, C. *et al.* (1987) EMBO J. **6**:109.
7. Ikuta, K. *et al.* (1987) Proc. Natl. Acad. Sci. USA **84**:819.
8. Kikutani, H. *et al.* (1986) Cell **47**:657.
9. Beavil, A.J. *et al.* (1992) Proc. Natl. Acad. Sci. USA **89**:753.
10. Kilmon, M.A. *et al.* (2004) J. Immunol. **172**:1065.
11. Beavil, R.L. *et al.* (1995) Immunology **84**:202.
12. Dierks, S.E. *et al.* (1993) J. Immunol. **150**:2372.
13. Yokota, A. *et al.* (1992) Proc. Natl. Acad. Sci. USA **89**:5030.
14. Yokota, A. *et al.* (1988) Cell **55**:611.
15. Montagnac, G. *et al.* (2005) J. Immunol. **174**:5562.
16. Karagiannis, S.N. *et al.* (2001) Immunology **103**:319.
17. Belleau, J.T. *et al.* (2005) Clin. Mol. Allergy **3**:6.
18. Letellier, M. *et al.* (1989) Mol. Immunol. **26**:1105.
19. Letellier, M. *et al.* (1988) J. Immunol. **141**:2374.
20. Sarfati, M. *et al.* (1992) Immunology **76**:662.
21. Marolewski, A.E. *et al.* (1998) Biochem. J. **333**:573.
22. Weskamp, G. *et al.* (2006) Nat. Immunol. **7**:1293.
23. Schulz, O. *et al.* (1997) Eur. J. Immunol. **27**:584.
24. Tu, Y. *et al.* (2005) Gastroenterology **129**:928.
25. Li, H. *et al.* (2006) Gastroenterology **131**:47.
26. Montagnac, G. *et al.* (2005) Traffic **6**:230.
27. Kilmon, M.A. *et al.* (2001) J. Immunol. **167**:3139.
28. Hibbert, R.G. *et al.* (2005) J. Exp. Med. **202**:751.
29. Aubry, J-P. *et al.* (1992) Nature **358**:505.
30. Sarfati, M. and G. Delespeese (1988) J. Immunol. **141**:2195.
31. Armant, M. *et al.* (1995) J. Immunol. **155**:4868.
32. Lecoanet-Henchoz, S. *et al.* (1995) Immunity **3**:11.
33. Hermann, P. *et al.* (1999) J. Cell Biol. **144**:767.
34. Schwarzmeier, J.D. *et al.* (2005) Leuk. Lymphoma **46**:157.
35. Schwarzmeier, J.D. *et al.* (2002) Leuk. Lymphoma **43**:549.
36. Poole, J.A. *et al.* (2005) J. Allergy Clin. Immunol. **116**:780.
37. Ribbens, C. *et al.* (2000) Clin. Exp. Immunol. **120**:194.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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