# Quantikine™ ELISA

## Human CCL24/Eotaxin-2 Immunoassay

Catalog Number DCC240B

For the quantitative determination of human Eotaxin-2 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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**Manufactured and Distributed by:**

**USA** R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413
**TEL:** 800 343 7475 612 379 2956
**FAX:** 612 656 4400
**E-MAIL:** info@bio-techne.com

**Distributed by:**

**Europe | Middle East | Africa** Bio-Techne Ltd.
19 Barton Lane, Abingdon Science Park
Abingdon OX14 3NB, UK
**TEL:** +44 (0)1235 529449
**FAX:** +44 (0)1235 533420
**E-MAIL:** info.emea@bio-techne.com

**China** Bio-Techne China Co., Ltd.
Unit 1901, Tower 3, Raffles City Changning Office,
1193 Changning Road, Shanghai PRC 200051
**TEL:** +86 (21) 52380373 (400) 821-3475
**FAX:** +86 (21) 52371001
**E-MAIL:** info.cn@bio-techne.com

# INTRODUCTION

Eotaxin-2, a CC chemokine also known as CCL24, MPIF-2, or Ckβ-6, was initially discovered through a large-scale cDNA sequencing effort of an activated monocyte library (1), and was independently characterized by three separate groups reporting in 1997 (1-3). Eotaxin-2 selectively recruits and activates eosinophils and basophils both *in vitro* (1-4) and *in vivo* (5), and signals via CC chemokine receptor 3 (CCR3). Like Eotaxin, Eotaxin-2 is a potential contributor to conditions featuring eosinophilia such as allergic reactions and parasitic infections (for a review of Eotaxin, see reference 6).

Mature Eotaxin-2 is a glycosylated 93 amino acid (aa) residue protein generated following removal of a 26 aa signal peptide. It has a predicted molecular weight of 10.6 kDa. Eotaxin-2 exhibits relatively low aa identity (32%-40%) with other known CC chemokines having overlapping functional profiles (*i.e.* Eotaxin, MCP-3, MCP-4, and RANTES) (3). The 3-dimensional structure of Eotaxin-2 confirms the existence of an N-loop structure important for receptor binding and an N-terminal α-helix implicated in receptor signaling (7). Eotaxin-2 and Eotaxin-3 are the only known chemokines to map to chromosome 7 (8-9).

Cross-desensitization (2) and receptor neutralization (4, 10, 11) studies indicate that Eotaxin-2 signals mainly, if not exclusively, through CCR3. CCR3 is a genetically polymorphic seven-transmembrane-spanning G-protein-linked receptor expressed on eosinophils, basophils, subpopulations of Th2 lymphocytes, and keratinocytes (12). Signal transduction via CCR3 is characterized by actin polymerization, a transient rise in cytosolic calcium concentration, and release of reactive oxygen species. Other chemokines capable of signaling through CCR3 include Eotaxin, MCP-2, MCP-3, MCP-4, MIP-1d, and RANTES.

Eotaxin-2 recruits and activates eosinophils and basophils with potency equal to that of Eotaxin (1-4). An *in vitro* inhibitory effect on multi-potent hematopoietic precursors has also been observed (1). Eotaxin-2 mRNA is expressed in activated T lymphocytes (1), GM-CSF treated macrophages (1), and dermal fibroblasts (13). In asthmatics, Eotaxin-2 is expressed by cytokeratin$^+$ epithelial cells, CD31$^+$ endothelial cells, and CD6$^+$ macrophages (14). Elevated Eotaxin-2 transcript has also been observed in tissue from nasal polyps (15). There is evidence that Eotaxin-2, along with MCP-4, is expressed at a later stage of eosinophilia than Eotaxin (16). The hookworm, a parasitic helminth, secretes metalloproteases that cleave and inactivate Eotaxin, but not Eotaxin-2 (17). This observation, along with differential chromosomal location, suggests that Eotaxin-2 may have evolved as a countermeasure against certain parasitic defense mechanisms capable of evading Eotaxin-induced reactions.

The Quantikine™ Human CCL24/Eotaxin-2 Immunoassay is a 4.5 hour solid phase ELISA designed to measure human Eotaxin-2 levels in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant human Eotaxin-2 and antibodies raised against the recombinant factor. Results obtained for naturally occurring human Eotaxin-2 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 natural human Eotaxin-2.

## PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human Eotaxin-2 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Eotaxin-2 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human Eotaxin-2 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 Eotaxin-2 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 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 Eotaxin-2 Microplate** | 893628 | 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human Eotaxin-2. | 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 Eotaxin-2 Conjugate** | 890942 | 21 mL of a polyclonal antibody specific for human Eotaxin-2 conjugated to horseradish peroxidase with preservatives. | May be stored for up to 1 month at 2-8 °C.* |
| **Human Eotaxin-2 Standard** | 893630 | Recombinant human Eotaxin-2 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.* | |
| **Assay Diluent RD1W** | 895117 | 11 mL of a buffered protein base with preservatives. | |
| **Calibrator Diluent RD5-5** | 895485 | 21 mL of a buffered protein base with preservatives. *For cell culture supernate samples.* | |
| **Calibrator Diluent RD6-35** | 895360 | 21 mL of animal serum with preservatives. *For serum/plasma 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 2N 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
- 500 mL graduated cylinder
- Test tubes for dilution of standards
- Human Eotaxin-2 Controls (optional; R&D Systems®, Catalog # QC22)

## PRECAUTIONS

Calibrator Diluent RD6-35 contains sodium azide which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

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.*
*Grossly hemolyzed samples are not recommended for use in this assay.*

## REAGENT PREPARATION

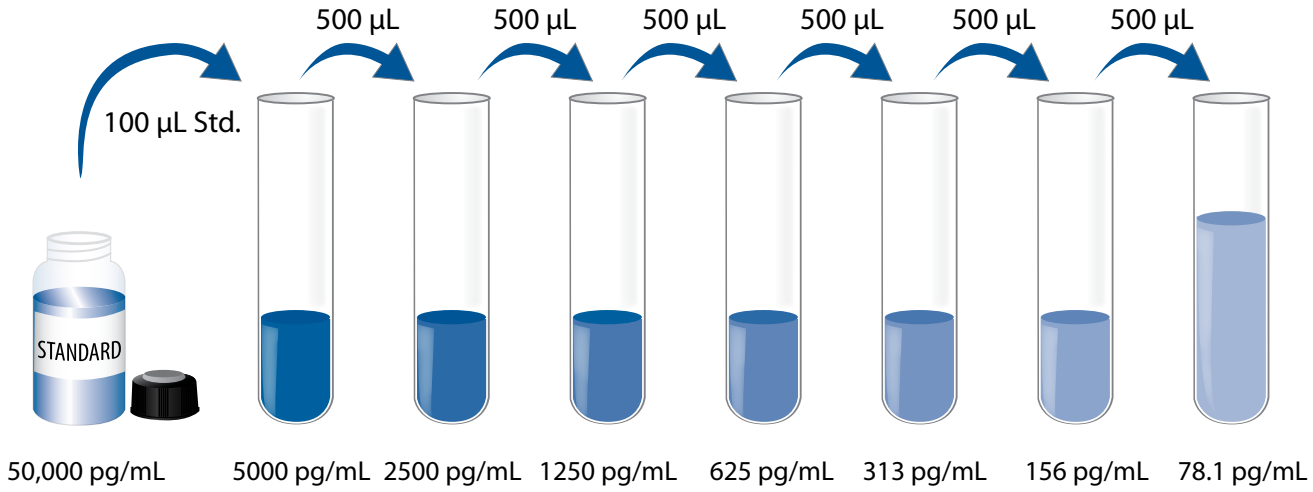
**Bring all reagents to room temperature before use.**

**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 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. 200 µL of the resultant mixture is required per well.

**Human Eotaxin-2 Standard** - **Refer to the vial label for reconstitution volume.** Reconstitute the Human Eotaxin-2 Standard with deionized or distilled water. This reconstitution produces a stock solution of 50,000 pg/mL. Allow the standard to sit for at least 15 minutes with gentle agitation prior to making dilutions.

Pipette 900 µL of Calibrator Diluent RD5-5 (*for cell culture supernate samples*) or Calibrator Diluent RD6-35 (*for serum/plasma samples*) into the 5000 pg/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 5000 pg/mL standard serves as the high standard. The appropriate calibrator diluent 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, controls, and samples 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 RD1W to each well.
4. Add 100 µL of standard, control, or sample per well. Cover with the adhesive strip provided. Incubate for 2 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 toweling.
6. Add 200 µL of Human Eotaxin-2 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. Protect from light.
   **For cell culture supernate samples:** Incubate for 20 minutes at room temperature.
   **For serum/plasma samples:** Incubate for 30 minutes at room temperature.
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.).

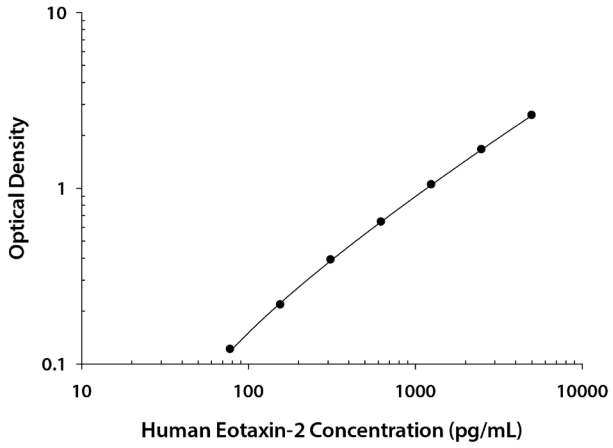
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human Eotaxin-2 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

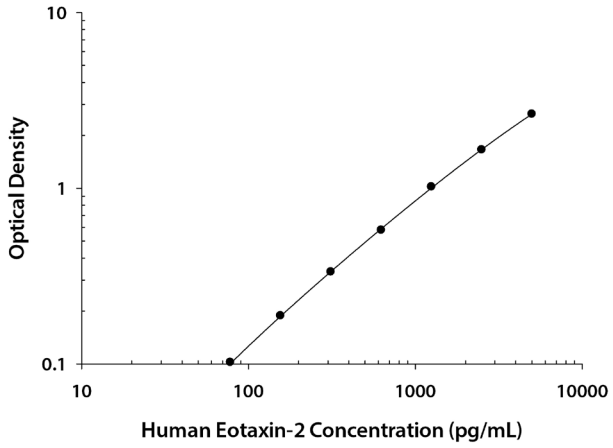
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

### CELL CULTURE SUPERNATE ASSAY




| (pg/mL) | O.D. | Average | Corrected |
|---|---|---|---|
| 0 | 0.012<br>0.013 | 0.013 | — |
| 78.1 | 0.133<br>0.135 | 0.134 | 0.121 |
| 156 | 0.228<br>0.232 | 0.230 | 0.217 |
| 313 | 0.404<br>0.405 | 0.404 | 0.391 |
| 625 | 0.655<br>0.656 | 0.655 | 0.642 |
| 1250 | 1.052<br>1.069 | 1.060 | 1.047 |
| 2500 | 1.652<br>1.693 | 1.672 | 1.659 |
| 5000 | 2.550<br>2.669 | 2.609 | 2.596 |

### SERUM/PLASMA ASSAY




| (pg/mL) | O.D. | Average | Corrected |
|---|---|---|---|
| 0 | 0.011<br>0.012 | 0.012 | — |
| 78.1 | 0.112<br>0.116 | 0.114 | 0.102 |
| 156 | 0.199<br>0.201 | 0.200 | 0.188 |
| 313 | 0.339<br>0.354 | 0.346 | 0.334 |
| 625 | 0.587<br>0.592 | 0.589 | 0.577 |
| 1250 | 1.029<br>1.032 | 1.030 | 1.018 |
| 2500 | 1.624<br>1.709 | 1.666 | 1.654 |
| 5000 | 2.604<br>2.706 | 2.655 | 2.643 |

## 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.

### CELL CULTURE SUPERNATE ASSAY

| | Intra-Assay Precision | | | Inter-Assay Precision | | |
|---|---|---|---|---|---|---|
| **Sample** | 1 | 2 | 3 | 1 | 2 | 3 |
| **n** | 20 | 20 | 20 | 40 | 40 | 40 |
| **Mean (pg/mL)** | 453 | 1239 | 2487 | 523 | 1379 | 2528 |
| **Standard deviation** | 43 | 100 | 188 | 51.4 | 74.6 | 141 |
| **CV (%)** | 9.6 | 8.0 | 7.6 | 9.8 | 5.4 | 5.6 |

### SERUM/PLASMA ASSAY

| | Intra-Assay Precision | | | Inter-Assay Precision | | |
|---|---|---|---|---|---|---|
| **Sample** | 1 | 2 | 3 | 1 | 2 | 3 |
| **n** | 20 | 20 | 20 | 40 | 40 | 40 |
| **Mean (pg/mL)** | 646 | 1856 | 3693 | 750 | 1971 | 3506 |
| **Standard deviation** | 46 | 121 | 201 | 63.2 | 112 | 284 |
| **CV (%)** | 7.1 | 6.5 | 5.4 | 8.4 | 5.7 | 8.1 |

## RECOVERY

The recovery of human Eotaxin-2 spiked to levels throughout the range of the assay in various matrices was evaluated.

| Sample Type | Average % Recovery | Range |
|---|---|---|
| Cell culture media (n=4) | 78 | 72-84% |
| Serum (n=4) | 103 | 84-116% |
| EDTA plasma (n=4) | 104 | 82-117% |
| Heparin plasma (n=4) | 99 | 80-115% |

## SENSITIVITY

One hundred fifty-five assays were evaluated and the minimum detectable dose (MDD) of human Eotaxin-2 ranged from 0.52-14.3 pg/mL. The mean MDD was 2.5 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.

## LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human Eotaxin-2 were diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

| | | Cell culture media (n=6) | Serum (n=8) | EDTA plasma (n=7) | Heparin plasma (n=7) |
|---|---|---|---|---|---|
| 1:2 | Average % of Expected | 108 | 95 | 91 | 95 |
| | Range (%) | 102-117 | 89-106 | 84-97 | 85-105 |
| 1:4 | Average % of Expected | 108 | 92 | 89 | 90 |
| | Range (%) | 95-120 | 80-101 | 77-96 | 76-103 |
| 1:8 | Average % of Expected | 111 | 95 | 90 | 91 |
| | Range (%) | 106-126 | 79-102 | 85-96 | 75-98 |
| 1:16 | Average % of Expected | 121 | 85 | 91 | 89 |
| | Range (%) | 116-131 | 66-93 | 91-91 | 86-93 |

## CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant human Eotaxin-2 produced at R&D Systems®.

## SAMPLE VALUES

**Serum/Plasma** - Samples from apparently healthy volunteers were evaluated for the presence of human Eotaxin-2 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) | 992 | 235-3106 | 595 |
| EDTA plasma (n=35) | 692 | 158-1927 | 418 |
| Heparin plasma (n=35) | 836 | 179-2257 | 512 |

**Cell Culture Supernates** - Human peripheral blood mononuclear cells (5 x $10^6$ cells/mL) were cultured in DMEM supplemented with 5% fetal bovine serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 units/mL penicillin, and 100 µg/mL streptomycin sulfate. The cells were cultured unstimulated or stimulated with 10 µg/mL PHA. Aliquots of the culture supernate were removed on days 1 and 6 and assayed for levels of natural human Eotaxin-2.

| Condition | Day 1 (pg/mL) | Day 6 (pg/mL) |
|---|---|---|
| Unstimulated | 602 | 4384 |
| Stimulated | ND | 5896 |

ND=Non-detectable

## SPECIFICITY

This assay recognizes natural and recombinant human Eotaxin-2.

Each of the factors listed below were prepared at 50 ng/mL in calibrator diluent and assayed. Preparations of the following factors at 50 ng/mL in a mid-range recombinant human Eotaxin-2 control were assayed for interference. No significant cross-reactivity or interference was observed.

### Recombinant human:

6Ckine
CCL28
CTACK
Eotaxin
Eotaxin-3
HCC-1
HCC-4
I-309
LKN-1
MCP-1
MCP-2
MCP-3
MCP-4
MDC
MIP-1α (66 aa)
MIP-1α (70 aa)
MIP-1β
MIP-1δ (68 aa)
MIP-1δ (92 aa)
MIP-3α
MIP-3β
MPIF-1
PARC
RANTES
TARC
TECK

### Recombinant mouse:

6Ckine
CTACK
Eotaxin
Eotaxin-2
JE/MCP-1
MARC
MCP-5
MDC
MIP-1α
MIP-1β
MIP-1γ
MIP-3α
MIP-3β
MPIF-2
RANTES
TARC
TECK
TCA-3

### Recombinant rat:

MIP-3α

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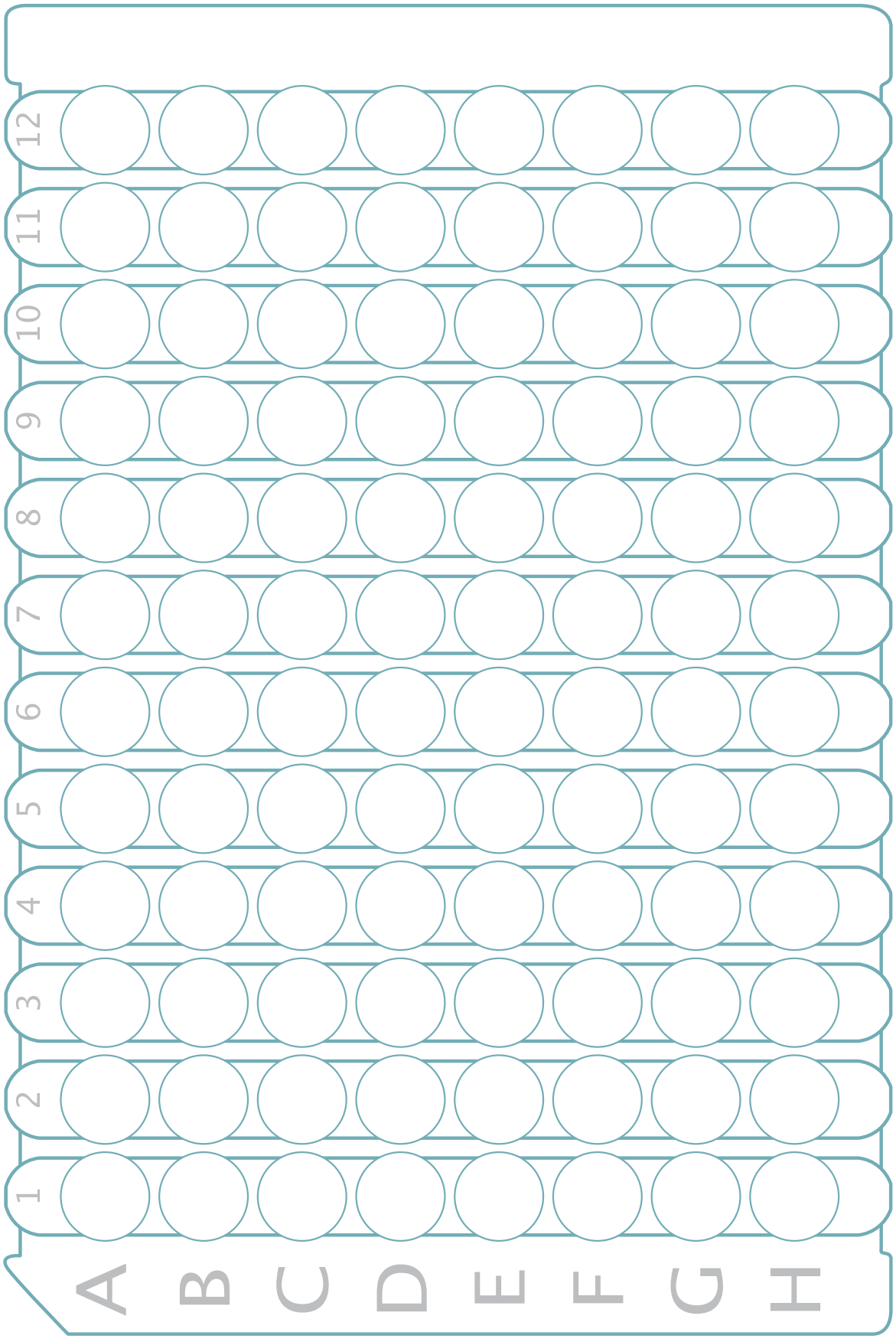
## PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



# NOTES

## NOTES