

# Quantikine<sup>®</sup>

## Mouse CCL17/TARC Immunoassay

Catalog Number MCC170

**For the quantitative determination of mouse thymus and activation-regulated chemokine (TARC) concentrations in cell culture supernates, mouse 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

Thymus and activation-regulated chemokine (TARC/CCL17) is a member of the CC or  $\beta$ -chemokine family (1, 2). Mouse TARC cDNA encodes a highly basic 93 amino acid (aa) residue precursor protein with a 23 aa residue putative signal peptide that is cleaved to generate the 70 aa residue secreted protein. The mature protein is not glycosylated and has a predicted molecular weight of 8.0 kDa (1). Mouse TARC shares 64% and 83% aa sequence identity with human and rat TARC, respectively (3, 4). Among other  $\beta$ -chemokine family members, TARC is most closely related to MDC, sharing approximately 33% aa sequence homology (5). TARC mRNA is constitutively expressed at high levels in thymic dendritic cells and at lower levels in lymph node dendritic cells in the lung, colon and small intestine (1, 5). Additional cell types that have been shown to express TARC include keratinocytes (6), monocytes (7), CD4<sup>+</sup> T cells (8), fibroblasts (9), bronchial epithelial cells (10), and Reed-Sternberg cells (11).

The chemokine receptor CCR4 has been shown to be a high-affinity functional receptor for TARC (12). Mouse and human CCR4 share 85% aa sequence identity (13, 14). In humans, CCR4 expression can be detected on Th2 CD4<sup>+</sup> T cells (12, 15), basophils (13), IL-2 activated NK cells (16), and platelets (17). Although CCR8 has also been reported to bind TARC, this finding is controversial (18, 19).

Recombinant TARC has been shown to chemoattract T cell lines and CCR4-transfected cells (5). Mouse TARC has no chemotactic activity on naive peripheral CD4<sup>+</sup> T cells but is a chemoattractant for memory/effector CD4<sup>+</sup> T cells with a preference for Th2 cells (20). One of the principal functions of TARC may be to recruit effector/memory T helper cells to antigen presenting dendritic cells at sites of inflammation (20). TARC has been shown to selectively recruit skin-homing CLA<sup>+</sup> memory CD4<sup>+</sup> T cells into skin during times of immune challenge (21). TARC has been shown to chemoattract IL-2 activated NK cell (16) and to induce platelet aggregation and degranulation (17).

The Quantikine Mouse TARC Immunoassay is a 4.5 hour solid phase ELISA designed to measure mouse TARC levels in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant mouse TARC and antibodies raised against the recombinant factor. Results obtained for naturally occurring mouse TARC showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that this immunoassay can be used to determine relative mass values for natural mouse TARC.

## **PRINCIPLE OF THE ASSAY**

This assay employs the quantitative sandwich enzyme immunoassay technique. A polyclonal antibody specific for mouse TARC has been pre-coated onto a microplate. Standards, Control, and samples are pipetted into the wells and any mouse TARC present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse TARC is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a Substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the Stop Solution is added. The intensity of the color measured is in proportion to the amount of mouse TARC bound in the initial step. The sample values are then read off the standard curve.

## **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 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.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

## PRECAUTION

The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

## 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.
- For best results, pipette reagents and samples into the center of each well.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- 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.

## MATERIALS PROVIDED

**Mouse TARC Microplate** (Part 890933) - One 96 well microplate (12 strips of 8 wells) coated with polyclonal antibody specific for mouse TARC.

**Mouse TARC Conjugate** (Part 890934) - 12.5 mL of a polyclonal antibody against mouse TARC conjugated to horseradish peroxidase with preservatives.

**Mouse TARC Standard** (Part 890935) - 2.5 ng of recombinant mouse TARC in a buffered protein base, with preservatives; lyophilized.

**Mouse TARC Control** (Part 890936) - 1 vial of recombinant mouse TARC in a buffered protein base with preservatives; lyophilized. The expected range of the mouse TARC control is shown on the vial label.

**Assay Diluent RD1W** (Part 895038) - 12.5 mL of a buffered protein solution with preservatives.

**Calibrator Diluent RD6-12** (Part 895214) - 21 mL of diluted animal serum with preservatives.

**Wash Buffer Concentrate** (Part 895024) - 50 mL of a 25-fold concentrated solution of a buffered surfactant with preservatives.

**Color Reagent A** (Part 895000) - 12.5 mL of stabilized hydrogen peroxide.

**Color Reagent B** (Part 895001) - 12.5 mL of stabilized chromogen (tetramethylbenzidine).

**Stop Solution** (Part 895174) - 23 mL of a diluted hydrochloric acid solution.

**Plate Covers** (Part 640197) - 4 adhesive plate sealers.

## STORAGE

|   |   |  |
|---|---|--|
| <b>Unopened Kit</b>                           | Store at 2 - 8° C. Do not use beyond kit expiration date. |  |
| <b>Opened/<br/>Reconstituted<br/>Reagents</b> | Mouse TARC Conjugate                                      | May be stored for up to 1 month at 2 - 8° C.*  |
|   | Diluted Wash Buffer                                       |  |
|   | Stop Solution   |  |
|   | Calibrator Diluent RD6-12                                 |  |
|   | Assay Diluent RD1W  |  |
|   | Unmixed Color Reagent A                                   |  |
|   | Unmixed Color Reagent B                                   |  |
|   | Mouse TARC Standard (500 pg/mL)                           | Aliquot and store for up to 1 month at ≤ -20° C in a manual defrost freezer.*  |
|   | Mouse TARC Control  |  |
|   | Microplate Wells  | Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2 - 8° C.* |

\*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.
- 1000 mL graduated cylinder.
- **Polypropylene** test tubes for serial dilution.

## SAMPLE COLLECTION AND STORAGE

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

**Serum** - Allow blood samples to clot for 2 hours at room temperature or overnight at 2 - 8° C before centrifuging for 20 minutes at 2000 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 as an anticoagulant. Centrifuge for 20 minutes at 2000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

**Note:** *Grossly hemolyzed or lipemic samples may not be suitable for measurement of mouse TARC with this assay.*

## REAGENT PREPARATION

Bring all reagents to room temperature before use.

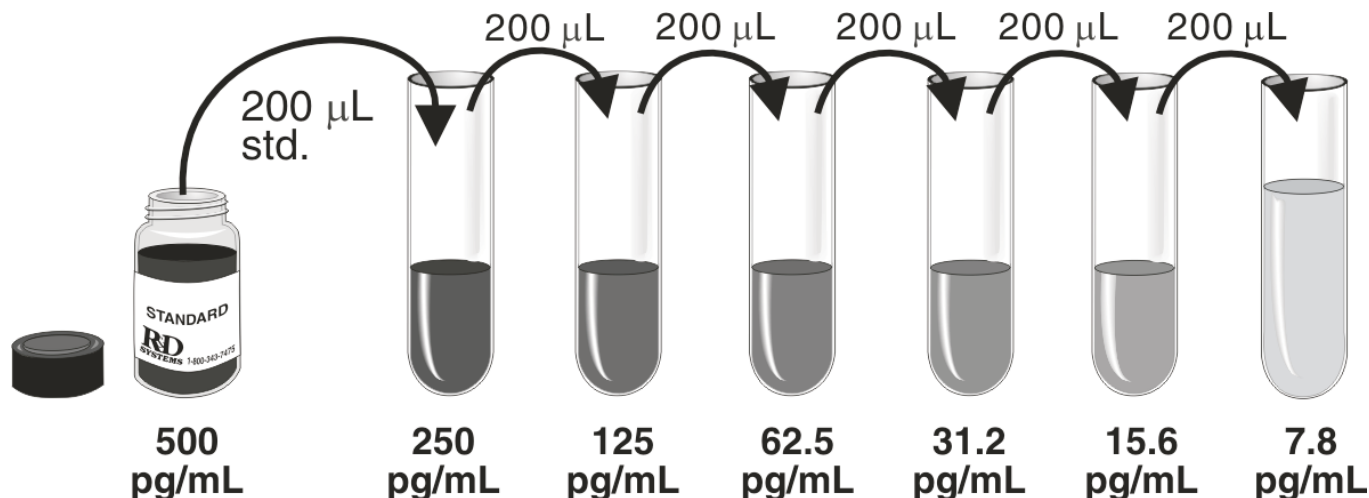
**Mouse TARC Kit Control** - Reconstitute the control with 1.0 mL of deionized or distilled water. Assay the Control undiluted.

**Wash Buffer** - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. To prepare enough Wash Buffer for one plate, add 25 mL Wash Buffer Concentrate into deionized or distilled water to prepare 625 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  $\mu$ L of the resultant mixture is required per well.

**Mouse TARC Standard** - Reconstitute the Mouse TARC Standard with 5.0 mL of Calibrator Diluent RD6-12. Do not substitute other diluents. This reconstitution produces a stock solution of 500 pg/mL. Allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

**Use polypropylene test tubes.** Pipette 200  $\mu$ L of Calibrator Diluent RD6-12 into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted mouse TARC Standard serves as the high standard (500 pg/mL). Calibrator Diluent RD6-12 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 samples, standards, and control be assayed in duplicate.**

1. Prepare reagents and standard dilutions 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  $\mu\text{L}$  of Assay Diluent RD1W to each well.
4. Add 50  $\mu\text{L}$  of Standard, Control, or sample per well. Mix by gently tapping the plate frame for 1 minute. 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 four times for a total of five washes. Wash by filling each well with Wash Buffer (400  $\mu\text{L}$ ) 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  $\mu\text{L}$  of 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 100  $\mu\text{L}$  of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
9. Add 100  $\mu\text{L}$  of Stop Solution to each well. 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.

## PROCEDURE SUMMARY AND CHECKLIST

1.  Bring all reagents to room temperature.  
 Prepare reagents and samples as instructed.  
 Return unused components to storage temperature as indicated in the instructions.
2.  Add 50  $\mu\text{L}$  Assay Diluent to each well.
3.  Add 50  $\mu\text{L}$  Standard, Control, or sample to each well.  
 Tap plate gently for one minute.  
 Cover the plate and incubate for 2 hours at room temperature.
4.  Aspirate and wash each well five times.
5.  Add 100  $\mu\text{L}$  Conjugate to each well.  
 Cover the plate and incubate for 2 hours at room temperature.
6.  Aspirate and wash each well five times.
7.  Add 100  $\mu\text{L}$  Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
8.  Add 100  $\mu\text{L}$  Stop Solution to each well.
9.  Read Optical Density at 450 nm (correction wavelength set at 540 nm or 570 nm).

## CALCULATION OF RESULTS

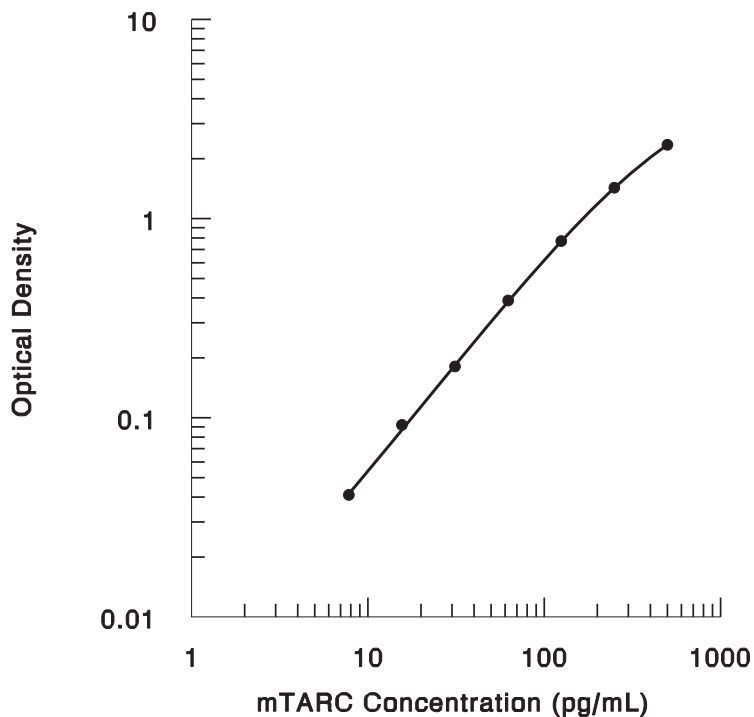
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density.

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 mouse TARC 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 prior to assay, the concentration read from the standard curve must be multiplied by the respective 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       | 0.051<br>0.054<br>0.094 | 0.052   | —         |
| 7.8     | 0.092<br>0.144          | 0.093   | 0.041     |
| 15.6    | 0.145<br>0.235          | 0.144   | 0.092     |
| 31.2    | 0.231<br>0.435          | 0.233   | 0.181     |
| 62.5    | 0.445<br>0.807          | 0.440   | 0.388     |
| 125     | 0.839<br>1.458          | 0.823   | 0.771     |
| 250     | 1.504<br>2.271          | 1.481   | 1.429     |
| 500     | 2.527                   | 2.399   | 2.347     |

## 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 assays to assess inter-assay precision.

| Sample             | Intra-assay Precision |     |     | Inter-assay precision |     |      |
|--------------------|-----------------------|-----|-----|-----------------------|-----|------|
|                    | 1                     | 2   | 3   | 1                     | 2   | 3    |
| n                  | 20                    | 20  | 20  | 20                    | 20  | 20   |
| Mean (pg/mL)       | 27                    | 89  | 278 | 29                    | 98  | 310  |
| Standard deviation | 1.3                   | 2.3 | 7.8 | 2.2                   | 6.9 | 17.3 |
| CV (%)             | 4.8                   | 2.6 | 2.8 | 7.6                   | 7.0 | 5.6  |

## RECOVERY

The recovery of mouse TARC spiked to three levels throughout the range of the assay in various matrices was evaluated.

| Sample Type                   | Average % Recovery | Range     |
|-------------------------------|--------------------|-----------|
| Cell culture supernates (n=6) | 94                 | 85 - 113% |
| mouse serum (n=6)             | 94                 | 82 - 103% |
| mouse EDTA plasma (n=6)       | 94                 | 83 - 109% |

## LINEARITY

To assess the linearity of the assay, five or more samples containing and/or spiked with various concentrations of mouse TARC in each matrix were diluted with Calibrator Diluent RD6-12 and assayed. Results from typical sample dilutions are shown.

| Sample                  | Dilution | Observed (pg/mL) | Expected (pg/mL) | Observed x 100 / Expected |
|-------------------------|----------|------------------|------------------|---------------------------|
| Cell Culture Supernates | neat     | 311              |                  |                           |
|                         | 1:2      | 145              | 156              | 93%                       |
|                         | 1:4      | 75               | 78               | 96%                       |
|                         | 1:8      | 38               | 39               | 97%                       |
|                         | 1:16     | 19               | 19               | 100%                      |
| Mouse Serum             | spiked   | 367              |                  |                           |
|                         | 1:2      | 190              | 183              | 104%                      |
|                         | 1:4      | 92               | 92               | 100%                      |
|                         | 1:8      | 53               | 46               | 115%                      |
|                         | 1:16     | 23               | 23               | 100%                      |
| Mouse EDTA Plasma       | spiked   | 382              |                  |                           |
|                         | 1:2      | 202              | 191              | 106%                      |
|                         | 1:4      | 106              | 96               | 110%                      |
|                         | 1:8      | 49               | 48               | 102%                      |
|                         | 1:16     | 25               | 24               | 104%                      |

## SENSITIVITY

The minimum detectable dose (MDD) of mouse TARC is typically less than 5 pg/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.

## CALIBRATION

This immunoassay is calibrated against highly purified *E. coli*-expressed recombinant mouse TARC produced at R&D Systems. This recombinant form of mouse TARC contains 70 amino acid residues and has a predicted molecular mass of 7.9 kDa.

Based on total amino acid analysis, the absorbance of a 1 mg/mL solution of the *E. coli*-expressed recombinant mouse TARC at 280 nm was determined to be 1.07 A.U.

## SAMPLE VALUES

**Serum/Plasma** - Twenty samples were evaluated for detectable levels of mouse TARC in this assay.

| Sample            | Mean (pg/mL) | Standard Deviation (pg/mL) | Range (pg/mL) |
|-------------------|--------------|----------------------------|---------------|
| Mouse serum       | 70           | 30                         | 20 - 123      |
| Mouse EDTA plasma | 69           | 20                         | 32 - 100      |

### **Cell Culture Supernates -**

Mouse splenocytes ( $1 \times 10^6$  cells/mL) were cultured for 3 days in RPMI plus 10% fetal calf serum supplemented with 50  $\mu$ M  $\beta$ -mercaptoethanol and 10 ng/mL rhIL-2. The cell culture supernate was assayed for mouse TARC and measured 246 pg/mL.

Mouse lung conditioned media (2 lungs, 1-2 mm pieces in 40 mL of medium) were collected after culturing for 6 days in RPMI with 10% fetal calf serum. The cell culture supernate was assayed for mouse TARC and measured 421 pg/mL.

Rat splenocytes ( $2 \times 10^6$  cells/mL) were cultured for 48 hours in DMEM plus 10% fetal calf serum and stimulated with 10 ng/mL PMA and 1  $\mu$ g/mL calcium ionomycin. The cell culture supernate was assayed for rat TARC and measured 340 pg/mL. Naturally occurring rat TARC from rat splenocytes showed linear dose curves that were parallel to the mouse standard curve.

## SPECIFICITY

This assay recognizes both recombinant and natural mouse TARC. The factors listed below were prepared at 50 ng/mL in Calibrator Diluent RD6-12 and assayed for cross-reactivity. Preparations of the following factors prepared at 50 ng/mL in a mid-range mouse TARC control were assayed for interference. No significant cross-reactivity or interference was observed.

### Recombinant mouse:

|               |           |                |               |
|---------------|-----------|----------------|---------------|
| C10           | IL-4      | IL-18          | P/IGF-2       |
| Eotaxin       | IL-5      | JE/MCP-1       | RANTES        |
| Flt-3 Ligand  | IL-6      | KC             | SCF           |
| G-CSF         | IL-7      | LIF            | TNF- $\alpha$ |
| GM-CSF        | IL-9      | MARC           | sTNF RI       |
| IFN- $\gamma$ | IL-10     | MCP-5          | sTNF RII      |
| IL-1 $\alpha$ | IL-10 sR  | M-CSF          | Tpo           |
| IL-1 $\beta$  | IL-12 p40 | MIP-1 $\alpha$ | TRANCE        |
| IL-1ra        | IL-12 p70 | MIP-2          | VEGF          |
| IL-2          | IL-13     | OPG            | VEGF R1       |
| IL-3          | IL-17     | OSM            |               |

Some cross-reactivity was observed with the following:

| Recombinant Factor | Concentration Tested (pg/mL) | Observed Value (pg/mL) | % Cross-reactivity |
|--------------------|------------------------------|------------------------|--------------------|
| Human TARC         | 50,000                       | 67                     | 0.1                |

Twenty rat serum and plasma samples were tested in this assay.

| Sample          | Mean (pg/mL) | Standard Deviation (pg/mL) | Range (pg/mL) |
|-----------------|--------------|----------------------------|---------------|
| Rat serum       | 13           | 7                          | 3 - 29        |
| Rat EDTA plasma | 10           | 4                          | 5 - 18        |

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# PLATE LAYOUT

Use this plate layout to record standards and samples assayed.

|    |   |   |   |   |   |   |   |   |
|----|---|---|---|---|---|---|---|---|
| 1  |   |   |   |   |   |   |   |   |
| 2  |   |   |   |   |   |   |   |   |
| 3  |   |   |   |   |   |   |   |   |
| 4  |   |   |   |   |   |   |   |   |
| 5  |   |   |   |   |   |   |   |   |
| 6  |   |   |   |   |   |   |   |   |
| 7  |   |   |   |   |   |   |   |   |
| 8  |   |   |   |   |   |   |   |   |
| 9  |   |   |   |   |   |   |   |   |
| 10 |   |   |   |   |   |   |   |   |
| 11 |   |   |   |   |   |   |   |   |
| 12 |   |   |   |   |   |   |   |   |
|    | A | B | C | D | E | F | G | H |