

Measuring Mast Cell Mediator Release

UNIT 7.38

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ABSTRACT

Mediators released from activated mast cells are responsible for the allergic inflammatory reactions associated with disease states such as anaphylaxis and atopy. These mediators are released as a consequence of immediate degranulation and phospholipid metabolism upon mast cell activation, followed by delayed cytokine gene expression. Thus, techniques that monitor indices of these events in mast cell culture systems, in association with biochemical analysis of parameters of cell signaling, are critical to our understanding of the molecular mechanisms regulating mast cell-mediated disease. Furthermore, these systems can be adapted for high-throughput screens to identify potential inhibitors of mast cell activation that may provide potential leads for novel therapies for these diseases. In this unit, we describe approaches that can be readily used or adapted to a variety of rodent and human mast cell culture systems for the determination of degranulation, phospholipid-derived inflammatory mediator production, and cytokine generation. *Curr. Protoc. Immunol.* 91:7.38.1-7.38.9. © 2010 by John Wiley & Sons, Inc.

Keywords: cytokines • degranulation • eicosanoids • FcεRI • human mast cells (HuMCs) • KIT • mouse bone marrow-derived mast cells (BMMCs) • stem cell factor (SCF).

INTRODUCTION

This unit presents protocols for measuring mediator release from both rodent and human mast cell cultures. These protocols can be used in conjunction with those described for the generation and isolation of rodent mast cells and mast cell lines (UNIT 3.23) and human mast cells and mast cell lines (see UNITS 7.25 & 7.37). Basic Protocol 1 is designed to monitor mast cell degranulation through the measurement of the release of the granule component β-hexosaminidase; Basic Protocol 2 is intended for determining the generation of products of phospholipid metabolism, the eicosanoids, leukotriene C₄ (LTC₄), and prostaglandin D₂ (PGD₂); and Basic Protocol 3 describes procedures for determining the generation of multiple cytokines.

MEASUREMENT OF MAST CELL DEGRANULATION AS MONITORED BY β-HEXOSAMINIDASE RELEASE

**BASIC
PROTOCOL 1**

β-Hexosaminidase is a component of mast cell granules, the activity of which can be readily monitored by a relatively sensitive colorimetric assay. Thus, monitoring the release of this enzyme is suitable for monitoring degranulation in a 96-well format.

NOTE: The genetic background of the mice influences the extent of FcεRI-mediated mast cell mediator release. For this reason it is essential to use the same genetic background, ideally littermate controls, of knockout and wild-type for direct comparisons.

Materials

C57BL/6J mice (The Jackson Laboratory) for obtaining BMMC
BMMC-MC/9 medium (UNIT 3.23)
CD34⁺-peripheral blood progenitor cells or human LAD2 mast cell line (see
UNIT 7.37) for obtaining HuMC

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Human mast cell culture medium or LAD2 cell culture medium (*UNIT 7.37*)
 Dinitrophenyl-human serum albumin conjugate (DNP-HSA) (Sigma-Aldrich)
 Human myeloma IgE (Calbiochem), biotinylated using NHS-biotin (Zymed); see
UNIT 5.3, Support Protocol 2
 Mouse monoclonal anti-DNP-IgE (clone SPE-7; Sigma-Aldrich)
 HEPES buffer, pH 7.4 (see recipe)
 Stimulants [e.g., dinitrophenyl-human serum albumin conjugate (DNP-HSA;
 Sigma-Aldrich) for BMMC or streptavidin for HuMC] or inhibitors of interest
p-nitrophenyl *N*-acetyl- β -D-glucosamide (PNAG; Sigma-Aldrich)
 Citrate buffer, pH 4.5: 40 mM citric acid/20 mM Na₂HPO₄·7 H₂O
 0.1% (v/v) Triton X-100
 400 mM glycine, pH 10.7
 Centrifuge: e.g., Sorvall RT-6000B refrigerated centrifuge
 96-well plates
 Heating block or warm air oven
 Spectrophotometer with microtiter plate reader
 Additional reagents and equipment for isolation and culture of mouse mast cells
 (*UNIT 3.23*) and human mast cells (*UNIT 7.37*)

NOTE: All solutions and equipment coming into contact with living cells must be sterile, and aseptic technique should be used accordingly.

NOTE: All incubations are performed in a humidified 37°C, 5% CO₂ incubator unless otherwise specified.

NOTE: All solutions are made in distilled water. All solutions and media must be filtered and kept at 4°C.

Prepare cells

HuMCs, LAD2 cells, and BMMCs are nonadherent cells. To replace medium or wash the cells, spin the cells 5 min at 450 × *g*, room temperature and carefully remove the supernatant; treat the cells gently.

- 1a. For BMMCs: Flush bone marrow cells from the femurs of C57BL/6J mice (*UNIT 3.23*), then culture the cells for 4 to 6 weeks in BMMC-MC/9 medium (see *UNIT 3.23*).

In some cases, BMMCs are cultured in SCF and IL-3 in RPMI. In this case, the results may be different from the cells cultured in IL-3 only, as in this protocol.

BMMCS can also be obtained from other mouse strains by this protocol. BMMCs are generally harvested for used between 4 to 6 weeks of culture.

- 1b. Develop primary HuMCs from CD34⁺-peripheral blood progenitor cells by culturing in human mast cell culture medium (containing IL-3 for first week of culture only; *UNIT 7.37*). Use after 7 to 10 weeks of culture. Alternatively, culture LAD2 in LAD2 cell culture medium (*UNIT 7.37*) and use up to 6 months after thawing.
2. When cells are ready to be used, to avoid the influence of IL-3, or SCF where included, starve cells 4 hr to overnight in cytokine-free medium. Sensitize cells by incubating overnight with an optimal concentration (100 ng/ml) of biotinylated human IgE (for HuMCs or LAD2 cells) or mouse anti-DNP-IgE (for BMMCs) added to the appropriate medium.

Prepare adequate numbers of cells for duplicate assays and to anticipate procedural losses.

3. Following sensitization, wash the cells three times with 10 ml HEPES buffer, pH 7.4 (maintained at 37°C), to remove excess IgE.

See text above step 1 of this protocol for washing conditions.

Perform assay

4. Resuspend cells in the HEPES buffer, pH 7.4, and aliquot the cells into 96-well plates (HuMCs or LAD2 cells at 5,000 to 10,000/well; BMMCs at 30,000 to 50,000/well).

Final volume per well is 100 μ l including agonist. As agonists and antagonists are added in 10 μ l of 10 $\times$ final concentration, cell suspensions are adjusted to accommodate this volume. For example, if the cells are treated with one agonist, the cell concentration is adjusted such that the correct final amount of cells is added in 90 μ l. If the cells are challenged with one agonist and one antagonist, then the cells are added in 80 μ l.

Samples must be run at least in duplicate. To correct for background fluorescence, prepare blanks (HEPES buffer only).

5. Incubate cells at 37°C for 5 to 10 min to equilibrate the cells at the activation temperature.
6. Prepare 10 $\times$ stocks of agonists or inhibitors in HEPES buffer. Then, add 10 μ l of these stocks to each well. For example, to stimulate BMMCs with 100 ng/ml of DNP-HSA, prepare 1 μ g/ml of DNP-HSA in HEPES buffer, and add 10 μ l into 90 μ l of the cell suspension in the well.

For inhibitor studies, cells are preincubated with inhibitors (e.g., wortmannin, LY294002) for 10 to 30 min prior to the addition of the agonists. When using SCF or other agonist such as a G-protein coupled receptor (GPCR) agonist, add just prior to, or concurrently with antigen.

7. Incubate the plate in a heating block or warm air oven at 37°C for 30 min (without CO₂).
8. During incubation, prepare *p*-nitrophenyl *N*-acetyl- β -D-glucosamide (PNAG) solution in citrate buffer. Calculate the total volume required based on a volume of 10 ml per complete 96-well plate. Dissolve 35 mg of PNAG per 10 ml of citrate buffer.

PNAG is poorly soluble in the citrate buffer; therefore sonication (using, e.g., a Cole-Parmer Ultrasonic Homogenizer) may be required for full solubilization.

9. Aliquot 100 μ l/well of the PNAG solution into two new 96-well plates using a multichannel pipettor.

One plate is for measurement of secreted β -hexosaminidase activity (supernatants) and the other is used to calculate the total amount (supernatants and lysates) to determine the percentage release.

10. After the incubation in step 7, spin the plate 5 min at 450 $\times$ g, 4°C, to stop the reaction and to ensure that the cells are sedimented to the base of the wells.
11. Tilt the plate to an angle of 45°, then CAREFULLY remove 50- μ l aliquots of the cell-free supernatant using a multichannel pipettor. Remove the liquid from the top of where the depth of the solution is greatest. Add the aliquots into the PNAG solution and incubate the plate for 90 min at 37°C (without CO₂; see step 7).

Make sure the cells are not removed and then transferred into PNAG solution when you take the 50 μ l of supernatant, as this will produce an artificially high degranulation value.

12. In order to calculate the total β -hexosaminidase activity, add 150 μ l/well of 0.1% Triton X-100 solution to the 50 μ l of supernatants and cells remaining in the wells of the original incubation plate. Resuspend carefully, take 50 μ l of the resulting lysate, add to the PNAG solution, and incubate the plate for 90 min at 37°C (without CO₂;

see step 7). Alternatively, add 150 µl of distilled water instead of 0.1% Triton X-100 solution and freeze the plate for >1 hr at –80°C. Defrost, mix by pipetting up and down, then remove 50 µl to assess lysate content as described above.

13. After incubation, add 50 µl 400 mM glycine to each well.

The appearance of yellow color indicates β-hexosaminidase activity.

14. Read plate absorbance at 405 nM with reference filter at 620 nm.

15. Calculate the percentage of β-hexosaminidase activities present in the supernatants.

a. Percentage degranulation = $100 \times (\text{supernatant content})/(\text{supernatant} + \text{lysate content})$.

b. However, only one half of the supernatant is assayed and plate blank needs to be deducted.

Therefore numerator (total supernatant reading) = $2 \times (\text{supernatant} - \text{plate blank})$.

c. Only one fourth of lysate is used and lysates still contain one half of supernatant. Plate blank also needs to be deducted.

Therefore denominator = (total cell content prior to stimulation) = $[0.5 \times (\text{total supernatant} - \text{plate blank})] + [4 \times (\text{lysates} - \text{plate blank})]$.

d. Therefore, % release = $100 \times [2 \times (\text{supernatant} - \text{plate blank})]/[0.5 \times (\text{total supernatant} - \text{plate blank}) + 4 \times (\text{lysates} - \text{plate blank})]$.

For example if supernatant reading = 0.4, lysates = 0.3, and plate blank = 0.04; then, % release = $100 \times (2 \times (0.4 - 0.04) / (0.4 - 0.04) + (4 \times (0.3 - 0.04))) = 51\%$.

These calculations can readily be carried out using a spread sheet such as MS-Excel.

BASIC PROTOCOL 2

MEASUREMENT OF EICOSANOID (PGD₂, LTC₄) GENERATION IN MAST CELLS

The eicosanoids PGD₂ and LTC₄ are generated from arachidonic acid by the cyclooxygenase and 5-lipoxygenase pathways, respectively. They are rapidly released from the cells following generation. Thus, their production can be readily monitored by assaying cell supernatants, following mast cell stimulation, by ELISA.

NOTE: PGD₂ is chemically unstable so it can rapidly degrade in the presence of serum. To avoid degradation, a PGD₂ methoxime enzyme immunoassay (PGD₂-MOX EIA) is used which is based on the conversion of PGD₂ to its stable MOX derivative.

Materials

Prostaglandin D₂-MOX EIA kit (e.g., Cayman Chemical)

Leukotriene C₄ EIA kit (e.g., Cayman Chemical)

96-well plate or 1.5-ml polypropylene microcentrifuge tubes

Additional reagents and equipment for measuring mast cell degranulation as monitored by β-hexosaminidase release (Basic Protocol 1)

NOTE: All solutions and equipment coming into contact with living cells must be sterile, and aseptic technique should be used accordingly.

NOTE: All incubations are performed in a humidified 37°C, 5% CO₂ incubator unless otherwise specified.

NOTE: Prepare ultrapure water for the eicosanoid measurement assay. Water must be deionized and free of trace organic contaminations. To accomplish the latter, use an activated carbon filter cartridge or organic scavengers.

1. Prepare HuMCs or BMMCs (Basic Protocol 1, step 1a or 1b). Sensitize overnight with an optimal concentration (100 ng/ml) of biotinylated human IgE or mouse SPE-7, respectively, in cytokine- and stem cell factor-free medium (see Basic Protocol 1, step 2).

Assays are performed in duplicate, so prepare an appropriate number of cells to account for this and also to account for procedural loss.

2. The next day, wash the cells three times with 10 ml of 37°C HEPES buffer, pH 7.4, to remove excess IgE.

See note over step 1 of Basic Protocol 1 regarding washing conditions.

3. Resuspend cells with HEPES buffer and aliquot the cells into wells of a 96-well plate or 1.5-ml microcentrifuge tubes (HuMCs, 200 to 250 cells/well or microcentrifuge tube for both PGD₂ and LTC₄; BMMCs, 2000 cells/well or tube for PGD₂, 100 cells/well or tube for LTC₄).

Final volume will be 100 µl/well or tube.

Cell number is very critical for measurement of antigen-induced eicosanoid generation. In the case of LTC₄ measurement in BMMCs, only 100 cells are needed. This low number can easily lead to inaccuracies in cell quantification. Therefore, it is recommended to use 100- to 1000-fold more cells and dilute the supernatants later with HEPES buffer.

To measure the generation of eicosanoids in response to other agonists such as SCF, GPCR agonist, etc, it may be necessary to first determine the optimal cell number.

4. Incubate cells at 37°C for 5 to 10 min to equilibrate the cells at the activation temperature, then add 1/10 of the final reaction volumes of a 10× stock of antigen in HEPES buffer (DNP-HSA for BMMCs or streptavidin for HuMCs; 10 to 100 ng/ml is the optimal concentration range).
5. Incubate the plate at 37°C for 20 to 30 min.
6. Spin down the plate or 1.5 ml microcentrifuge tube 5 min at 450 × g, 4°C, to recover cell-free supernatant. Store the supernatants at –80°C until assayed.

Eicosanoids are chemically unstable and can rapidly degrade. Samples should thus be assayed immediately after collection or stored at –80°C until assayed.

7. Measure LTC₄ and PGD₂ in the supernatants using a competitive enzyme immunoassay kit according to the manufacturer's instructions.

The amounts of LTC₄ and PGD₂ are calculated based on the standard curve (10 pg/ml to 1000 pg/ml). A new standard curve must be run for each assay, as results can vary depending on the development condition.

MEASUREMENT OF CYTOKINE RELEASE FROM MAST CELLS

Cytokines are generated following induction of gene expression. Thus, compared to degranulation and eicosanoid generation, a longer time of stimulation (4 to 8 hr) is required to detect measurable amounts released. Nevertheless, cytokines present in the cell supernatants can be readily measured by ELISA.

Materials

Cytokine assay kit (e.g., R&D systems)
24- or 48-well plate

Additional reagents and equipment for measuring mast cell degranulation as monitored by β-hexosaminidase release (Basic Protocol 1)

BASIC PROTOCOL 3

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7.38.5

NOTE: All solutions and equipment coming into contact with living cells must be sterile, and aseptic technique should be used accordingly.

NOTE: All incubations are performed in a humidified 37°C, 5% CO₂ incubator unless otherwise specified.

1. Prepare HuMCs or BMMCs (Basic Protocol 1, step 1a or 1b).
- 2a. Starve BMMCs of cytokines and sensitize overnight with an optimal concentration (100 ng/ml) of mouse anti-DNP IgE (SPE-7) as for degranulation (Basic Protocol 1, step 2).
- 2b. Sensitize HuMC overnight with 100 ng/ml of biotinylated human IgE in complete human mast cell culture medium (containing SCF and IL-6).

In the case of HuMCs, antigen or SCF alone fails to generate sufficient cytokine to be detected. To detect cytokine release, it is necessary to incubate the cells with antigen and SCF together.

3. The next day, wash BMMCs three times with 10 ml 37°C cytokine-free BMMC-MC/9 medium or wash HuMCs three times with 10 ml 37°C complete human mast cell culture medium, to remove excess IgE.
4. Resuspend BMMCs with cytokine-free medium and aliquot the cells into a 24- or 48-well plate (100 µl/well for 24-well plate or 500 µl/well for 48-well plate at 0.5×10^6 cells/ml). For HuMCs, resuspend the cells with complete medium and aliquot the cells into a 24- to 48-well plate (100 µl/well at 1×10^6 cells/ml).

Perform cytokine measurement at least in duplicate (100 µl/sample). Accordingly, prepare enough cells.

5. Prepare 10× stocks of antigen (DNP-HSA for BMMC; streptavidin for HuMCs) in the same medium.

For BMMC and HuMC, 10 to 100 ng/ml antigen concentration is optimal.

The human mast cell culture medium contains 100 ng/ml of SCF; therefore it is not necessary to add additional SCF.

6. Incubate cells 4 to 8 hr at 37°C.

In mast cells, antigen/SCF stimulation increases the mRNA and protein synthesis for multiple cytokines. Although it depends on the cytokine generated, generally 2 to 4 hr is the optimal time for detecting mRNA message and 4 to 8 hr is optimal for protein detection. For each new cytokine, it will be necessary to optimize the incubation time.

7. Pipet the supernatants into 1.5 ml microcentrifuge tubes and centrifuge 2 min at $5000 \times g$, 4°C, to prepare cell-free supernatants. Store the supernatants at –80°C until assayed.
8. Measure the cytokine content using an appropriate kit according to the manufacturer's instructions.

The amount of cytokine or chemokine is calculated based on the standard curve (32.5 pg/ml to 2000 pg/ml).

If the sample's OD is above the standard curve range, the samples will need to be diluted to 1/5 or 1/10.

REAGENTS AND SOLUTIONS

Use deionized, distilled water in all recipes and protocol steps. For common stock solutions, see APPENDIX 2A; for suppliers, see APPENDIX 5.

HEPES buffer (pH 7.4)

10 mM HEPES (10 mM)
137 mM NaCl
2.7 mM KCl
0.4 mM Na₂HPO₄·7H₂O
5.6 mM glucose
1.8 mM CaCl₂·2H₂O
1.3 mM MgSO₄·7 H₂O
0.4% (w/v) bovine serum albumin (BSA)
Store up to 6 months at 4°C

COMMENTARY

Background Information

Mast cell mediators likely evolved to participate in innate and adaptive defense mechanisms designed to protect the body against invading pathogens and parasites (Marshall, 2004; Brown et al., 2008). However, these mediators have the capacity to produce potentially life-threatening or otherwise disabling consequences in those within the general population who are susceptible to anaphylactic reactions and atopy (Metcalfe et al., 1997; Brown et al., 2008). These mediators are released upon mast cell activation following antigen-induced aggregation of the high-affinity receptors for IgE (FcεRI) following binding of the antigen to antigen-specific IgE occupying these receptors (Gilfillan and Tkaczyk, 2006). Multiple other receptors expressed on mast cells can, however, modify FcεRI-mediated mast cell activation (Hundley et al., 2004; Qiao et al., 2006; Kuehn and Gilfillan, 2007). The mediators released in this manner are diverse in both nature and function. They can, however, be categorized into three general groups based on their mode of generation and/or release.

The first group of mediators contains those that are pre-synthesized and stored in mast cell granules (Metcalfe et al., 1997; Blank and Rivera, 2006). The major such mediator with relevance to disease is histamine. However, mast cell granules also contain other bioactive amines such as serotonin, various proteases, carboxypeptidases, and other enzymes. Mast cell degranulation has been monitored by determining the extent of appearance of a number of these granule components in mast cell supernatants relative to the amount remaining in the tissues. The most widely used proto-

cols are those designed to measure the release of histamine, serotonin, or β-hexosaminidase. Of these, the simplest and cheapest, and the one most amenable to the 96-well format, is the β-hexosaminidase assay outlined in this unit.

The second group of mediators are the phospholipid metabolites, which are generated as a consequence of the activation of phospholipase (PL)A₂ upon mast cell activation. Two subgroups of mediators are generated in this manner: platelet activating factor and the eicosanoids—PGD₂, LTC₄, and LTB₄ (Boyce, 2007). Eicosanoids are generated from arachidonic acid, which is liberated primarily from arachidonyl-containing phosphatidylcholine by PLA₂. Thus, early studies looking at the generation and release of eicosanoids from mast cells adopted a protocol whereby the cells were incubated with radiolabeled arachidonic acid, followed by post-chasing and then determining the extent of label released following mast cell activation (UNIT 7.33). This technique has both advantages and disadvantages. The advantages include a detection of the overall range of eicosanoids released from activated mast cells. The disadvantages include the requirement for use of radioactivity or knowledge of the use of equipment such as high-performance liquid chromatography (HPLC). More recent methods have employed enzyme immunoassay (ELISA) kits and radioimmunoassay (RIA) specific for each eicosanoid. Both RIA and ELISA have high sensitivity, simplicity, and specificity. However, ELISA and RIA are designed to detect only one eicosanoid; therefore, a comprehensive investigation of eicosanoid generation can be expensive.

The final group of mediators released from activated mast cells are the cytokines and chemokines which are generated as a consequence of enhanced gene expression (Metcalf et al., 1997; Hundley et al., 2004; Gilfillan and Tkaczyk, 2006). The generation and/or release of these products has been assayed by multiple different approaches, both at the mRNA level and at the protein level. It should, however, be remembered that relative mRNA levels are not necessarily reflective of the amount of cytokine protein released from the cells. Messenger RNA levels for multiple cytokines can be simultaneously assayed either by using commercially available RNase protection assays or multiplex gene chips. Messenger RNA levels for individual cytokines can also be measured by RT-PCR or by semi-quantitative real-time PCR following design of the appropriate primers. Release of cytokines from activated mast cells is readily measured by ELISA in a similar manner to that for LTC₄ and PGD₂. However, the time of stimulation is appreciably longer (4 to 8 hr compared to less than 30 min) than that required for degranulation or eicosanoid generation.

Troubleshooting

If there is no color change or very little color in the β -hexosaminidase release assay, the viability of the cells may be compromised. If BMMC or HuMC cultures are older than 7 weeks or 10 weeks, respectively, β -hexosaminidase release will begin to decrease in comparison with younger cell cultures. Therefore, the cells should be used at 4 to 6 weeks (for BMMCs) or 7 to 10 weeks (for HuMCs). The background of β -hexosaminidase release should be less than 5%. If the background (without stimulation) is higher than 5%, this may be due to cells being removed along with the supernatants

following centrifugation. Alternatively, a high background may reflect the presence of IgE aggregates during the sensitization stage. To minimize this problem, avoid multiple freeze-thaw cycles and centrifuge the IgE at 14,000 $\times$ g at 4°C for 60 min, then collect the supernatant and store at 4°C. Normally, the IgE is stable for many months at this temperature.

Eicosanoids are lipid-mediated products; thus, all samples must be free of organic solvents prior to assay. If the water or buffers are contaminated with organic solvents, one may not see any color change. In this case, the source of ultrapure water should be changed or the solution should be filtered through an organic scavenger. If one sees a color change in the test samples but not the standard curve, the standard may be degraded. Eicosanoids are chemically unstable, so it is easy for them to rapidly degrade. In this case, prepare a new standard curve and test again.

If there is no color change in the cytokine measurement, it may be due to degradation of the samples. Repeated freezing and thawing can degrade cytokines. Thus, the samples and standard must be aliquotted before freezing (–80°C). If your sample's OD is outside of the standard curve, you need to increase cell number or dilute the samples properly.

Anticipated Results

For each assay, the appropriate number of cells, incubation times, and antigen concentrations are different. The anticipated amount of mediators released from mouse BMMCs and HuMCs are given in Tables 7.38.1 and 7.38.2, respectively.

When SCF is added concurrently with antigen, degranulation, cytokine, and chemokine increase 2- to 5-fold compared to that produced by antigen alone.

Table 7.38.1 Setup Conditions and Anticipated Amounts of Mediators Released in Mouse Mast Cell Mediator Release Assays

Assay for BMMCs	Cells per well/100 μ l	Incubation time	Antigen	Anticipated release
β -hexosaminidase	3-5 $\times$ 10 ⁴	30 min	10 ng/ml	20%-50%
LTC ₄	100	30 min	10 ng/ml	700-1200 pg/ml
PGD ₂	2 $\times$ 10 ³	30 min	10 ng/ml	90-200 pg/ml
Cytokine	1 $\times$ 10 ⁵	6 hr	10 ng/ml	IL-6: 300-600 pg/ml TNF α : 100-200 pg/ml
Chemokine	1 $\times$ 10 ⁵	6 hr	10 ng/ml	MCP-1 : 200-300 pg/ml MIP1- α : 400-800 pg/ml

Table 7.38.2 Setup Conditions and Anticipated Amounts of Mediators Released in Human Mast Cell Mediator Release Assays

Assay for HuMCs	Cells per well/100 μ l	Incubation time	Antigen	Anticipated release
β -hexosaminidase	$0.5-1 \times 10^4$	30 min	10 ng/ml	20%-60%
LTC ₄	200	30 min	10 ng/ml	100-130 pg/ml
PGD ₂	200	30 min	10 ng/ml	120-250 pg/ml
Cytokine	1×10^5	6 hr	100 ng/ml (option: SCF 100 ng/ml)	IL-8: 200-600 pg/ml GM-CSF: 50-100 pg/ml

Time Considerations

Sensitization of cells with IgE requires a minimum of 3 to 4 hr with a maximum of overnight. After sensitization, the subsequent washing steps, stimulation of cells with antigen, and recovery of supernatants and total cell lysates require 3 hr for all degranulation studies. For eicosanoid generation, ELISA requires 2 days (2 hr for sample preparation, 18 hr for plate incubation, and 2 hr for development of the plate). For cytokine measurement, sample preparation requires 4 to 8 hr. After collection of the sample, the ELISA typically takes 4 to 6 hr.

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