

# Label-Free Cellular Assay Enables Detection of Endogenous Chemokine Receptor Activity in Human T Lymphoblastoid Cells

CORNING  
Epic<sup>®</sup>  
technology



## SnAPPShots

A brief technical report  
from the Corning  
Development Group

*Alice Gao*  
Corning Incorporated,  
Life Sciences  
Kennebunk, ME 04043

## Introduction

Corning® Epic® label-free cell assay technology utilizes a resonant waveguide grating sensor to monitor the integrated cellular responses initiated by the activation of target proteins.<sup>1</sup> Such responses are the result of a series of biochemical interactions within a cell and involve the translocation of multiple molecules, referred to as dynamic mass redistribution (DMR).<sup>1</sup> The DMR signal obtained through the Epic technology is a novel quantifiable measurement, highly specific, and has been successfully used to monitor the activities of G-protein coupled receptors (GPCRs), ion channels and receptor tyrosine kinases.<sup>2,3,4</sup> In addition to the benefit of allowing the observation of target activation independent of pathways or mechanisms, DMR measurement through Epic technology also allows one to monitor the activities of endogenously expressed targets in a totally noninvasive environment, thus providing more physiologically relevant information.

In this study, a label-free assay was developed to investigate the activities of an endogenously-expressed chemokine receptor CCR4 in CCRF-CEM cells.

Chemokine receptors play a fundamental role in lymphocyte trafficking. They interact with chemokines produced at sites of infection or destruction and specific areas of lymphoid tissue, and direct the migration of lymphocytes towards these sites. Such recruitment can be a critical event that triggers and sustains the clinical manifestations of inflammation. Therefore, these receptors or their ligands have been implicated in the treatment for many inflammatory diseases.<sup>5,6</sup> Chemokine receptors have also been targeted in cancer therapy due to their elevated expression in certain types of tumors.<sup>7,8</sup> This study showed a robust CCR4 response to ligand stimulation. It was also demonstrated that neutralization effect by antibodies can also be studied using Epic label-free technology.

## Materials and Methods

### Reagents

All cell culture reagents and assay buffer components were purchased from Invitrogen (Carlsbad, CA). Recombinant mouse and human CCL17/TARC, the monoclonal anti-mouse CCL17 antibody, and the isotype antibody control (Rat IgG2A) were purchased from R&D Systems, Inc. (Minneapolis, MN, Cat. No. 529-TR, Cat. No. 364-DN, Cat. No. MAB529, and Cat. No. MAB006). Fatty-acid-free BSA was purchased from Sigma-Aldrich (St. Louis, MO, Cat. No. A8806). Corning polypropylene microplates (Cat. No. 3657) were used to prepare ligand solutions and fibronectin-coated Corning Epic microplates (Cat. No. 5042) were used for all assays performed in this study, except where specified.

### Cell Cultures

CCRF-CEM cell line used in this study was purchased from ATCC (Manassas, VA, Cat. No. CCL-119). Cells were cultured in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum (FBS) as recommended by the cell line supplier.

### Epic Assay Procedures

On the same day of performing the assay, fresh cells in flasks were harvested by centrifugation at 1000 rpm (200 x g) for 3 to 5 minutes. After the medium was discarded, the cell pellets were resuspended in assay buffer (HBSS containing 20 mM HEPES and 0.05% fatty acid free BSA). The resulting cell suspensions were used to seed Epic cell assay microplates in 30 µL volume. Except where specified, approximately 60,000 cells were seeded per well. After cell seeding, the microplates were allowed to equilibrate inside the Epic reader. Equilibration times of 1 to 3.5 hours were tested in order to find the optimal timing. Baseline signals were then measured followed by the addition of chemokines. The DMR response to chemokine addition was monitored immediately for 30 minutes. In the assays involving neutralizing antibodies, the chemokine was pre-incubated with the antibodies for 30 minutes before addition to the cells.

### Data Analysis

The response profile/traces were obtained using Epic Offline Viewer software. Dose responses and curve fitting were constructed using GraphPad Prism® software.

## Results and Discussion

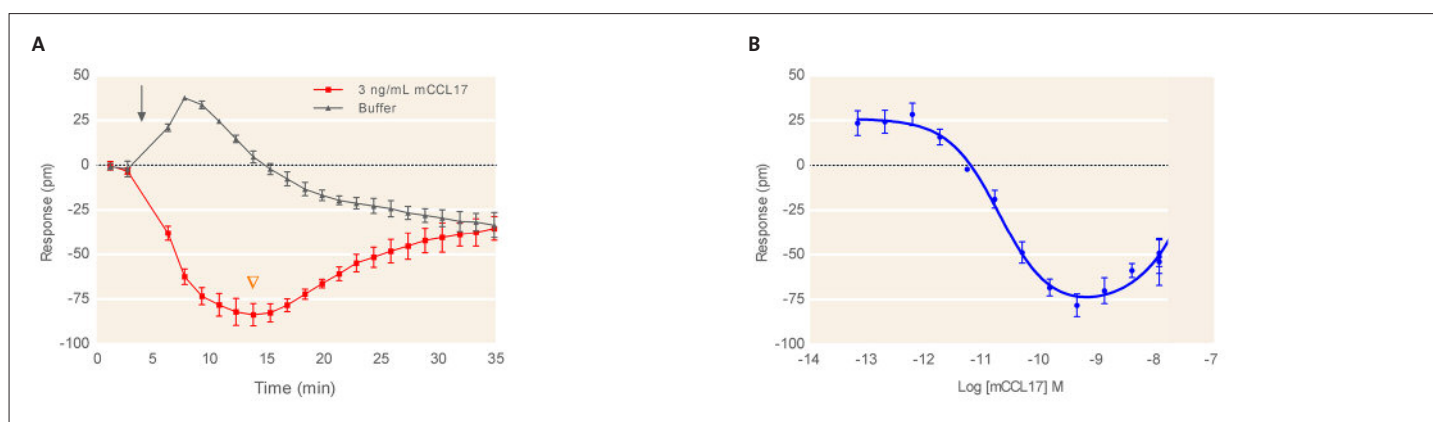
### mCCL17 Induced DMR Kinetics and Dose-dependency

Mouse CCL17 (mCCL17) induced a large negative DMR response in CCRF-CEM cells (Fig. 1A), which reached a maximum (negative dip) at about 10 minutes after stimulation. This response exhibited a bell-shaped dose dependency (Fig. 1B), which is one of the characteristics typical for leukocytes when migrating towards a chemotactic stimulus.<sup>9</sup> The activity of mCCL17 in the label-free assay format was also very potent, achieving maximal effect at approximately 3 ng/mL concentration. In a chemotaxis assay performed by the mCCL17 supplier, maximal migration activity of CCRF-CEM cells was achieved at 30 to 40 ng/mL ligand concentration. These results indicated a good correlation between the activities obtained by the traditional chemotaxis assay and the Epic label-free assay.

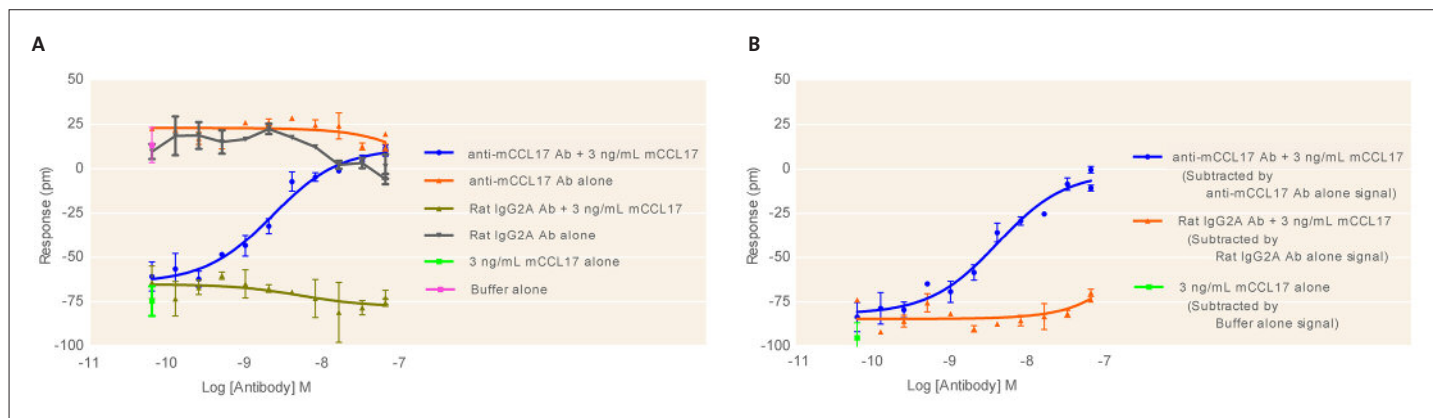
Human CCL17 (hCCL17) also induced a response similar to that of mCCL17 with reduced magnitude of ~60% (data not shown).

### Inhibition of mCCL17 Induced Response by Anti-mCCL17 Antibody

The effect of an anti-mCCL17 antibody was investigated first using varying concentrations of the antibody at a fixed 3 ng/mL mCCL17 concentration for maximal activity, followed by a fixed dose of 2 µg/mL antibody (equivalent to IC<sub>90</sub>) and varying concentrations of the ligand mCCL17. In the first set of experiments, the isotype antibody Rat IgG2A was used as a negative control. As shown in Fig. 2, the anti-mCCL17 antibody exhibited inhibitory effect upon the mCCL17-induced response with an IC<sub>50</sub> of ~2.3 nM which is equivalent to 350 ng/mL antibody. No effect was observed for the isotype antibody. Both antibodies alone did not induce any responses. These results suggest that the mCCL17-induced N-DMR response in CCRF-CEM cells is ligand-specific. In a chemotaxis assay performed by the antibody supplier using a cell line over-expressing CCR4, the anti-mCCL17 antibody showed an IC<sub>50</sub> near 750 to 3000 ng/mL, which is in agreement with the value obtained here.



**Figure 1.** Response of CCRF-CEM cells to stimulation of recombinant mouse CCL17/TARC (mCCL17). (A) DMR kinetic profiles. 3 ng/mL of mCCL17 is roughly equal to 0.3 nM concentration. Arrow indicates the addition of the chemokine or buffer. Orange triangle indicates the time where the values of the response were taken to construct the dose curve. (B) Dose-dependent (bell-shaped) response to mCCL17 stimulation. Maximal activity was observed at ~0.3 nM. The signals plotted here were the 10 minute responses after ligand stimulation. N=4.



**Figure 2.** Neutralization effect by a monoclonal anti-mCCL17 antibody, showing the specificity and dose dependency. Antibodies and mCCL17 were pre-incubated together for 30 minutes prior to use for dosing the cells. Top concentration for the antibodies tested were 66 nM which is equivalent to 10 µg/mL, assuming molecular weight of 150 kDa for the antibodies. (A) 10-minute signals after ligand or antibody addition. IC<sub>50</sub> for the neutralization effect was ~2.3 nM, equivalent to 350 ng/mL. (B) Control-subtracted signals. N=3.

The inhibitory effect of the anti-mCCL17 antibody was also evident in its ability to right-shift the potency for the stimulatory effect of mCCL17 (Fig. 3). In the absence of the antibody, maximal N-DMR was obtained at 0.3 nM of mCCL17. In the presence of the antibody, the ligand concentration for the maximal N-DMR was greater than 10 nM; more than 10-fold right shift. This effect was expected as the neutralizing antibody reduces the availability of active ligand in the environment. Again, no effect was observed for the negative control antibody Rat IgG2A.

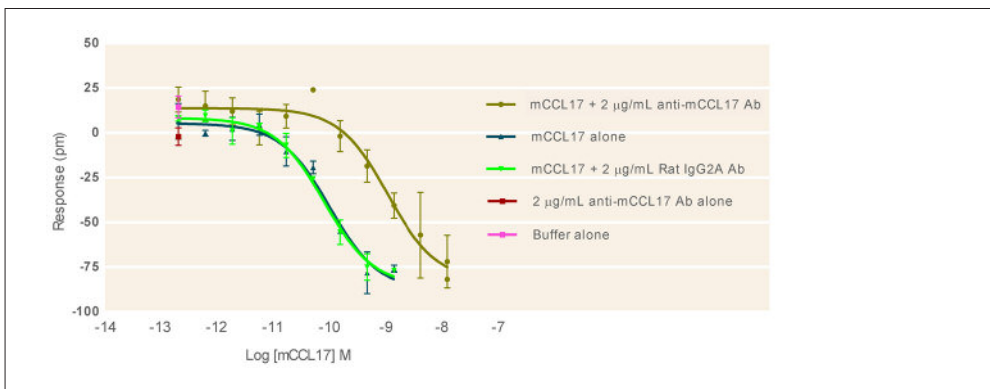
**Impact of Seeding Density, Buffer Incubation Time and Microplate Surface on Assay Performance**

In the process of optimizing the assay performance, several parameters were tested, including cell plating density, microplate surfaces and buffer equilibration time. As shown in

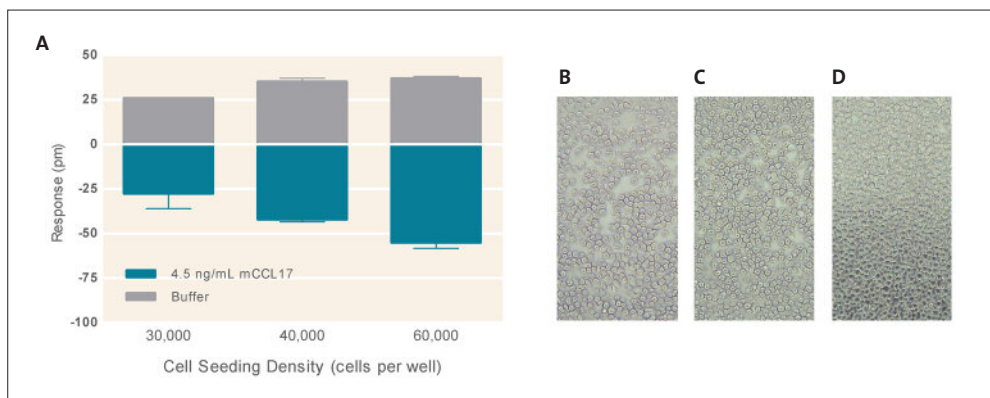
Fig. 4A, the best assay window (the difference between the buffer control signal and the maximal response induced by the ligand) was obtained at a plating density of 60,000 cells per well. This seeding density is commonly used for nonadherent cells in cell-based assays, and results in coverage greater than 90% of the surface in a well (Figure 4D).

On an uncoated surface at the same seeding density, cell coverage of the surface was similar to that on the Fibronectin surface (Fig. 5B versus Fig. 4D). However, maximal response obtained on the uncoated surface was much smaller than that obtained on the Fibronectin coated surface (Fig. 5A).

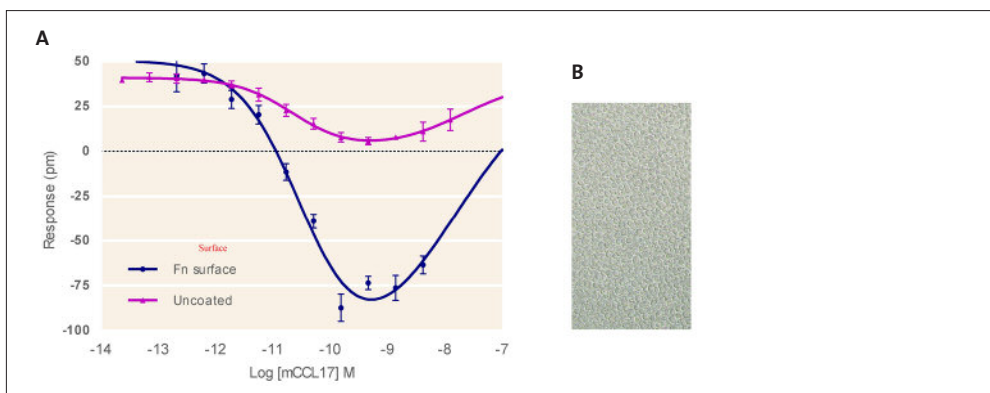
It was found that buffer equilibration time (the period between buffer-exchange and the start of the assay) also has an impact on the assay performance. After a 1 hour equilibration period, cells exhibited a higher response to



**Figure 3.** Neutralization effect by a monoclonal anti-mCCL17 antibody, showing a right-shift of the potency for mCCL17-induced response in CCRF-CEM cells from 0.3 nM to greater than 10 nM. Antibodies and mCCL17 were pre-incubated together for 30 minutes prior to use for dosing the cells. Antibody concentration used here was 2 µg/mL which is equivalent to 13.2 nM, assuming molecular weight of 150 kDa for the antibodies. Signals plotted here were 10 minute signals after ligand or antibody addition. N=3.



**Figure 4.** Comparison of seeding density effect on assay window. (A) Response to buffer and mCCL17 under different seeding densities. (B-D) Microscopic pictures showing the coverage of well bottom by the cells under different seeding densities. Pictures were taken 30 minutes after seeding. N=4.



**Figure 5.** Impact of surface on assay performance. Seeding density of 60,000 cells per well was used in this experiment. (A) Dose-dependent response to mCCL17 on Fibronectin (Fn) coated and uncoated surfaces. (B) Microscopic picture showing the coverage of well bottom by the cells on uncoated surface. Picture was taken 30 minutes after seeding. N=4.

buffer alone and smaller response to ligand stimulation when compared to the results with either a 2.3 or 3.3 hour equilibration time (Fig. 6A and Fig. 6B). As a result, the entire dose-response curve was elevated (Fig. 6C, green curve). However, the shape of the dose-response curve obtained under different equilibration periods was not altered (Fig. 6C).

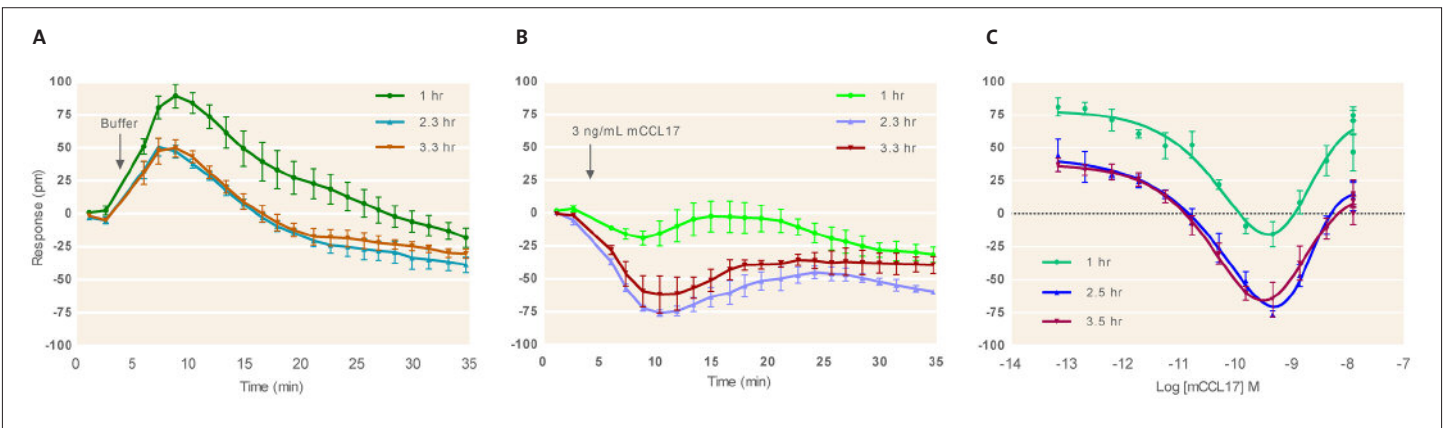
### Impact of BSA in Assay Buffer on Assay Performance

Inclusion of BSA in assay buffer is a common practice to prevent the loss of assay components to surfaces during sample preparation and during assays, due to nonspecific binding. Such surfaces include compound microplate and assay microplate surfaces, pipet tip surfaces and microcentrifuge tubes. In cell-based assays, especially in the situations where sticky ligands such as peptides are used, nonspecific binding can lead to reduced ligand potency. In biochemical assays, this issue can not only reduce ligand potency manifested as right-shifted  $K_d$ s, but also diminish enzyme activities, leading to poor assay results.

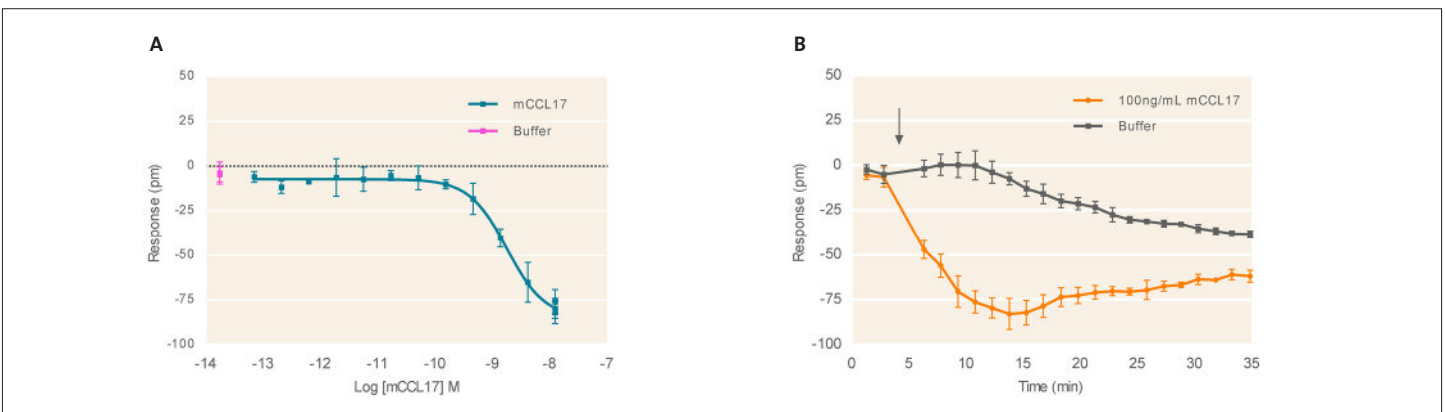
The impact of BSA in the assay buffer was evident for this assay. As shown by the dose-response curve in Fig. 7A, in the absence of BSA, mCCL17-induced N-DMR response did not reach a maximum until its concentration was increased to 10 nM, which was more than 10-fold higher than the concentration needed in assay buffer containing 0.05% BSA. The response to buffer addition, however, was minimal when BSA was excluded in the assay buffer. No significant difference was observed in the DMR profiles stimulated by mCCL17 in the absence or presence of BSA (comparing Fig. 7B to Fig. 1A).

### Conclusions

- ▶ The activities of the endogenously expressed chemokine receptor CCR4 in CCRF-CEM cells were detected using Epic® technology. The response signal was specific and the assay was robust.
- ▶ Neutralization effect from anti-ligand antibody was also detected using Epic technology with excellent specificity.



**Figure 6.** Impact of buffer equilibration time prior to performing assays. (A) Response to buffer addition. (B) Response to mCCL17 addition. (C) Comparison of dose-dependency to mCCL17 stimulation under different equilibration periods. N=4.



**Figure 7.** Impact of BSA in assay buffer on ligand potency. (A) DMR kinetic profiles. 100 ng/mL of mCCL17 is roughly equal to 10 nM concentration. Arrow indicates the addition of the chemokine or buffer. (B) Dose-dependent response to mCCL17 stimulation. Maximal activity was observed at >10 nM. The signals plotted here were the 10-minute responses after ligand stimulation. N=4.

## References

1. Ye, F. (2007) Non-invasive Optical biosensor for probing cell signaling. *Sensors* 7:2316-2329.
2. Ye, F. et al. (2008) Label-free cell-based assays for GPCR Screening. *Combinatorial Chemistry & High Throughput Screening* 11:357-369.
3. Ye, F. et al. (2005) Optical biosensors for monitoring dynamic mass redistribution in living cells mediated by epidermal growth factor receptor activation. *Proceedings of the 2005 IEEE*, September 1-4.
4. Peters, M.F. et al. (2010) Comparing label-free biosensors for pharmacological screening with cell-based functional assays. *Assay Drug Dev, Technol.* 8(2):219-227.
5. D'Ambrosio, D. et al (2003) Chemokine receptors in inflammation: an overview. *J. Immunol. Methods* 273(1-2):3-13.
6. Banfield, G. et al (2010) CC chemokine receptor 4 (CCR4) in human allergen-induced late nasal responses. *Allergy* 65(9):1126-1133.
7. Di Stasi, A. et al (2009) T lymphocytes coexpressing CCR4 and a chimeric antigen receptor targeting CD30 have improved homing and antitumor activity in a Hodgkin tumor model. *Blood* 113(25):6392-6402.
8. Vaday, G.G. et al. (2006) Expression of CCL5 (RANTES) and CCR5 in prostate cancer. *Prostate* 66(2):124-134.
9. Traves, S.L. and Donnelly, L.E. (2005) Chemokines and their Receptors as Targets for the Treatment of COPD. *Current Respir. Med. Rev.* 1:15-32.

For additional product or technical information, please visit [www.corning.com/lifesciences](http://www.corning.com/lifesciences) or call 1.800.492.1110. Outside the United States, please call 978.442.2200.

# CORNING

## **Corning Incorporated** *Life Sciences*

Tower 2, 4th Floor  
900 Chelmsford St.  
Lowell, MA 01851  
t 800.492.1110  
t 978.442.2200  
f 978.442.2476

[www.corning.com/lifesciences](http://www.corning.com/lifesciences)

## **Worldwide Support Offices**

**ASIA / PACIFIC**  
**Australia/New Zealand**  
t 0402-794-347

**China**  
t 86 21 2215 2888  
f 86 21 6215 2988

**India**  
t 91 124 4604000  
f 91 124 4604099

**Japan**  
t 81 3-3586 1996  
f 81 3-3586 1291

**Korea**  
t 82 2-796-9500  
f 82 2-796-9300

**Singapore**  
t 65 6733-6511  
f 65 6861-2913

**Taiwan**  
t 886 2-2716-0338  
f 886 2-2516-7500

## **EUROPE**

**France**  
t 0800 916 882  
f 0800 918 636

**Germany**  
t 0800 101 1153  
f 0800 101 2427

**The Netherlands**  
t 31 20 655 79 28  
f 31 20 659 76 73

**United Kingdom**  
t 0800 376 8660  
f 0800 279 1117

**All Other European  
Countries**  
t 31 (0) 20 659 60 51  
f 31 (0) 20 659 76 73

**LATIN AMERICA**  
**Brasil**  
t (55-11) 3089-7419  
f (55-11) 3167-0700

**Mexico**  
t (52-81) 8158-8400  
f (52-81) 8313-8589