

# Profiling Receptor Tyrosine Kinase Activity with Corning® Epic® Label-Free Technology

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## SnAPPShots

A brief technical report  
from the Corning  
Applications Group

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## Introduction

The epidermal growth factor receptor (EGFR) is a member of the type 1 receptor tyrosine kinase (RTK) family and a key regulator of development and growth in cells. As abnormal EGFR activity can result in oncogenic proliferation, EGFR and RTK family members have emerged as cancer biomarkers and therapeutic targets (1,2). RTK ligand binding leads to activation of receptor kinase activity and subsequent autophosphorylation of tyrosine residues in the cytosolic domain. The activated receptor can also phosphorylate other protein substrates, resulting in a signaling pathway dependent upon kinase activity. Label-free measurements using Corning Epic Technology provide a single assay platform for profiling RTK-dependent signaling pathways. The real-time optical response observed upon the binding of ligands to RTK receptors on the cell surface results from a redistribution of proteins within the cells, referred to as dynamic mass redistribution (DMR). Therefore, Corning Epic Technology can provide a more comprehensive view of the intracellular events that occur following RTK activation and inhibition. In addition, Corning Epic Technology is sensitive enough to detect cellular responses from endogenous receptors, alleviating the need to use engineered cell lines.

Corning Epic Technology has been used previously to measure RTK responses in an epidermoid carcinoma cell line (3). To extend this observation, we tested HeLa cells for endogenous EGF activity and profiled kinase involvement upon EGFR stimulation with a library of 80 kinase inhibitors. Our data show that endogenous EGFR responses can be measured using Corning Epic Technology and readily inhibited to profile signaling pathway kinase activity. Furthermore, modulation and potentiation of the EGFR response upon kinase inhibition provides insight into the downstream contributions of other signaling pathways in an endogenous EGFR response.

## Materials and Methods

### Cells

HeLa (Cat. No. CCL-2) cells were obtained from American Type Culture Collection (Manassas, VA). Cells were maintained in Eagle's Minimum Essential Medium (Cat. No. 10-010-CV) supplemented with 10% heat-inactivated Fetal Bovine Serum (Cat. No. 35-016-CV) and 2mM L-Glutamine (Cat. No. 25-005-C1). Assay buffer for measuring EGF response was Hank's Balanced Salt Solution (HBSS) (Cat. No. 14025) containing 20mM HEPES (Cat. No. 15630). Assay buffer for kinase library profiling also included 1% Dimethyl Sulfoxide (DMSO) (Cat. No. D4540). All cell culture reagents were purchased from Corning cellgro® (Manassas, VA) except for DMSO, HBSS and HEPES, which were purchased from Sigma-Aldrich (St. Louis, MO) and Invitrogen (Carlsbad, CA), respectively.

### Reagents

Epidermal Growth Factor (EGF) (Cat. No. E9644) and epinephrine (Cat. No. E4642) were purchased from Sigma-Aldrich. The Tocriscreen™ Kinase Inhibitor Toolbox (Cat. No. 3514) was obtained from Tocris Bioscience (Ellisville, MS).

## Epic Assay Procedures

### Cell Seeding

Frozen stock subcultures of HeLa cells were thawed into complete growth medium and counted. Cells were then seeded into Corning Epic 384 well Fibronectin-Coated Cell Assay Microplates (Cat. No. 5042) at 30K cells per well. All plates were seeded using a Thermo Scientific® Multidrop Combi cell culture dispensing system. The seeded microplates were allowed to sit at room temperature for 30 minutes to allow cells to uniformly settle on the growth surface of the well, then incubated for 18 to 22 hours in a 37°C/5% CO<sub>2</sub> humidified incubator prior to beginning the assay.

### Buffer Exchange

After overnight culture, the growth medium was replaced with assay buffer containing 1% DMSO using the Molecular Devices Aquamax® DW4 automated microplate washer. The plate was then stored in the Corning Epic instrument for 2 hours prior to obtaining a baseline measurement to allow the cells to recover from the wash procedure and enable thermal equilibration to occur.

## Reformatting the Tocriscreen™ Kinase Library

The Tocriscreen Kinase Inhibitor Toolbox was reformatted from a 96 well format into a 384 well format with each inhibitor present in quadruplicate using the Evolution™ P3 (Perkin Elmer). The 384 well source microplate, with 1  $\mu$ L of inhibitor per well at 2 mM in 100% DMSO, was reformatted with the same instrument to a 4X source microplate with 25  $\mu$ L of inhibitor per well at 40  $\mu$ M in 1% DMSO.

## Epic Label-Free Measurement

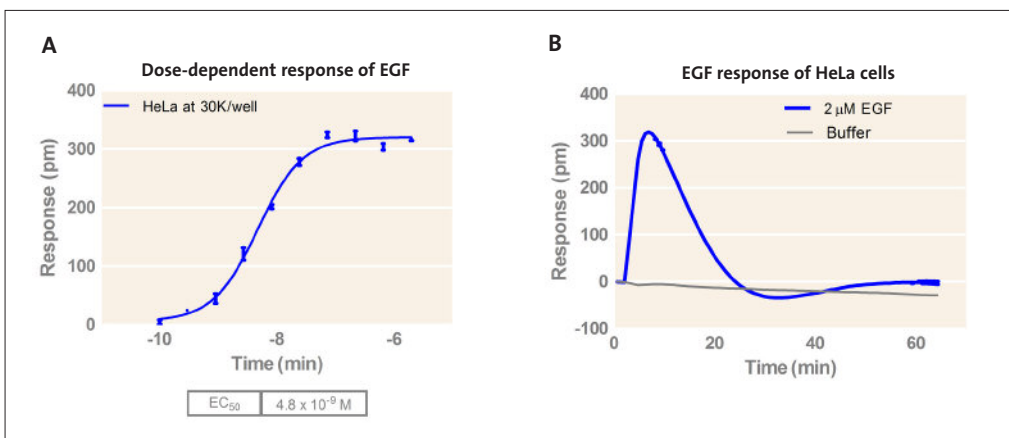
For initial EGF testing in HeLa cells, a 5 minute baseline measurement was taken after the assay microplate pre-equilibrated in the Corning® Epic® instrument. Dose-response of EGF and an appropriate control compound were added to the cells in assay buffer, and then a kinetic read was taken for 60 minutes. For kinase library assays, a baseline measurement was taken for 5 minutes, and then the kinase inhibitors were added to the cells in assay buffer, followed by an initial kinetic read of 60 minutes. Subsequently, EGF was added to the cells at an  $EC_{80}$  dose in assay buffer, and a second kinetic read of 60 minutes was performed. All compound additions were performed using a CyBio CyBi®-Well pipetting system.

## Data Analysis

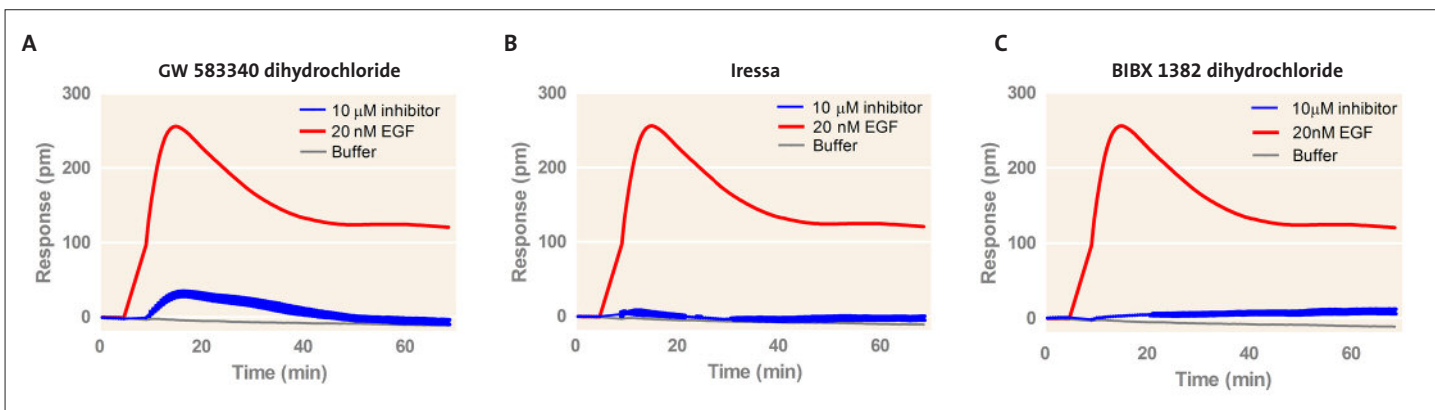
DMR response values were obtained using Corning Epic Data Analysis software. Dose response curves and optical response profiles were generated using Graphpad Prism® software.

## Results and Discussion

In order to investigate kinases involved in the EGFR response, we first looked at endogenous EGF activity in a panel of epithelial cell lines (data not shown). HeLa cells demonstrating the most robust response using saturating concentrations of EGF were then tested using a dose series of EGF, as shown in Fig. 1A. These cells showed a potent dose-dependent response to EGF, yielding an  $EC_{50}$  of 4.8nM (Fig. 1A). The DMR trace for the top dose of EGF can be visualized in Fig. 1B. HeLa cells exhibit an initial response that quickly rises upward peaking at approximately 10 minutes. This kinetic response in the positive direction is due to the net movement of mass (proteins and cytoskeletal reorganization) towards the sensing region, defined as positive dynamic mass redistribution (P-DMR). The response then transitions downward, plateauing around 30 minutes. This kinetic response in the negative direction is caused by a



**Figure 1.** Endogenous EGFR activity can be measured in HeLa cells. (A) HeLa cells exhibit dose-dependent response to EGF with an  $EC_{50}$  of 4.8 nM. (B) DMR response in HeLa cells upon stimulation with 2  $\mu$ M EGF shows peak P-DMR at approximately 10 minutes.



**Figure 2.** EGFR inhibitors can block endogenous EGF DMR responses in HeLa cells. All three EGF inhibitors in Tocris kinase library show significant inhibition of EGF response at  $EC_{80}$  dose of 20 nM. (A) GW 583340 dihydrochloride, a dual EGFR//Erb2 inhibitor inhibits approximately 90% of peak EGF response. (B,C) BIBX 1382 dihydrochloride and Iressa, selective EGFR inhibitors completely inhibit entire EGF signal.

net movement of mass away from the sensor region, defined as negative dynamic mass redistribution (N-DMR). Due to the robust endogenous response detected, we proceeded with kinase profiling of the EGFR response.

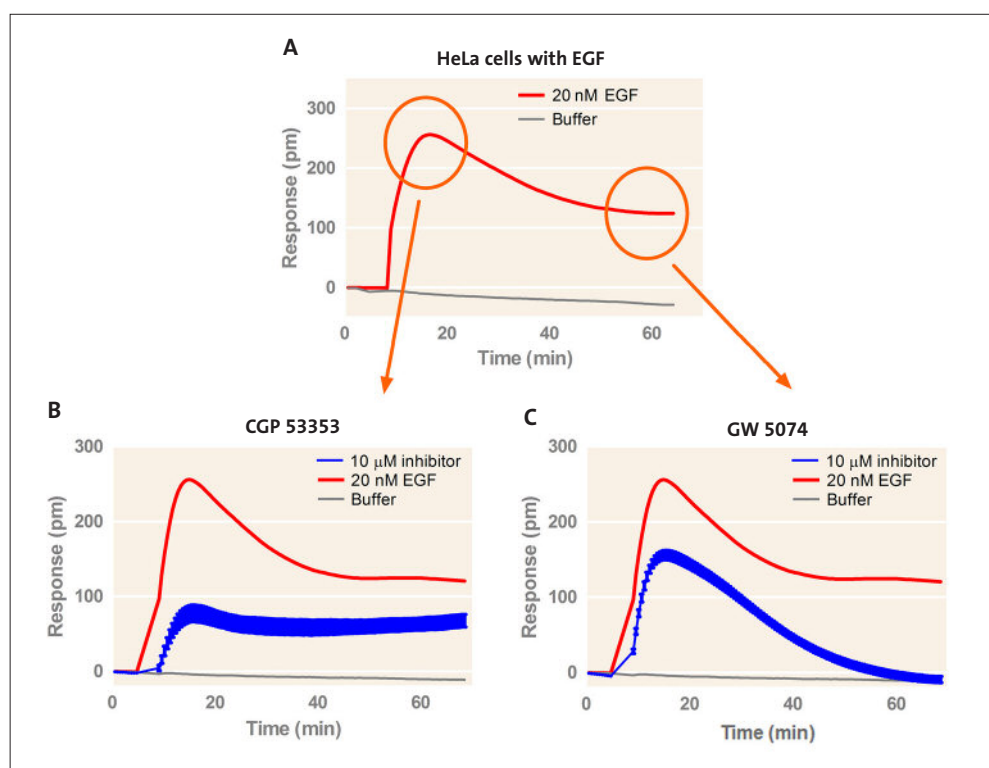
To evaluate downstream pathway biology following EGFR activation, we applied a library of 80 kinase inhibitors to HeLa cells prior to stimulation with EGF. As expected, we found that all three EGFR kinase inhibitors present in the library completely inhibited the observed DMR in HeLa cells upon activation with an EC<sub>80</sub> dose of EGF (20 nM), which is shown in Fig. 2. In addition, eleven kinase inhibitors were also identified that modulated the EGF response in HeLa cells. We classified these inhibitors according to the way in which they inhibited the EGF optical response profile (see Table 1). For example, two kinase inhibitors were identified that lowered the initial P-DMR peak at ~10 minutes by >60%, as shown in Fig. 3B. Six additional kinase inhibitors were identified that lowered the end response at ~60 minutes by >60%, as shown in Fig. 3C. Further, there were 3 kinase inhibitors that are not specific EGFR inhibitors that also lowered the DMR in response to EGF in HeLa cells by >60% at both the ~10 minute and ~60 minute time points. The DMR traces of these inhibitors are similar to the EGFR kinase inhibitors in Fig. 2 A-C. In summary, the 14 kinase inhibitors that were identified came from a variety of signaling pathways, including the EGFR pathway. This provides insight into contributions from other signaling pathways that influence the endogenous EGFR response in HeLa cells.

Along with identifying kinase inhibitors that lower the DMR in response to EGF in HeLa cells, we also detected

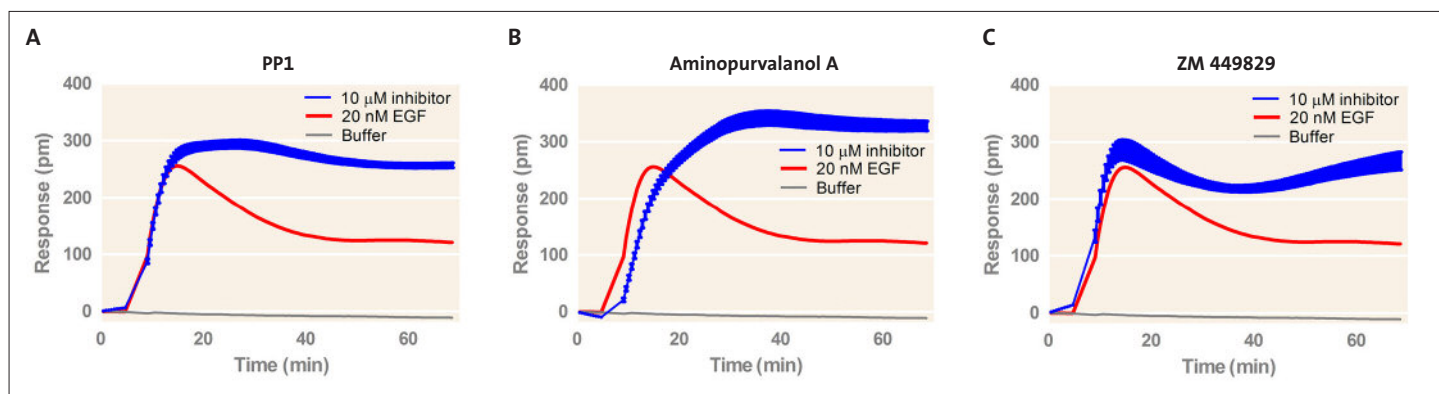
**Table 1. Kinase activity can be linked to temporal regions of the EGF optical trace.** Inhibitors that contribute to the entire DMR trace are shown in the top group. These inhibitors show >60% inhibition at both 10 and 60 minutes. The middle group is comprised of inhibitors that contribute to the peak response of EGF at 10 minutes. These inhibitors show >60% inhibition at 10 minutes and <60% inhibition at 60 minutes. The bottom group is comprised of inhibitors that contribute to the EGF response at 60 minutes. These inhibitors show >60% inhibition at 60 minutes and <60% inhibition at 10 minutes.

Inhibitor	Target
<b>Common at 10 and 60 minutes</b>	
BI 78D3	JNK
FPA 124	Akt/PKB
ZM 306416 hydrochloride	VEGF
<b>&gt;60% inhibition at 10 minutes only</b>	
CGP 53353	PKC II
BIO	GSK-3
<b>&gt;60% inhibition at 60 minutes only</b>	
GW 5074	c-Raf1
PD 407824	Chk1 and Wee1
GSK 650394	SGK
SB 218078	Chk1
Ro 31-8220 mesylate	Protein kinase inhibitor
Ryuvidine	Cdk4

several kinase inhibitors that potentiated the EGF response. These kinase inhibitors are listed in Table 2, and representative graphs depicting the altered kinetic traces are shown in Fig. 4. When these kinases are inhibited, the EGF response results in a larger DMR with an enhanced P-DMR peak at



**Figure 3.** The Optical trace of EGF can be broken down by investigating contribution of certain kinases. (A) 20 nM of EGF shows distinct optical traces in HeLa cells. (B) Initial P-DMR peak at approximately 10 minutes can be linked to kinases, such as CGP 53353, which inhibit >60% of the EGF response at 10 minutes but not at the 60 minute time point. (C) Plateauing response towards the end of the EGF response can be linked to kinases, such as GW 5074, which inhibit >60% of the EGF response at 60 minutes, not at the 10-minute time point.



**Figure 4.** Certain kinases show potentiation of EGF response in HeLa cells. (A) PP1 shows potentiation of EGF response following peak response at 10 minutes. (B) Aminopurvalanol A exhibits intensified signal along with a change in optical response profile when compared to the EGF response. (C) ZM 449829 exhibits overall potentiation throughout EGF optical response profile with increased potentiation towards the end of the EGF response.

~10 minutes, or a prolonged response that persists without the characteristic N-DMR. Potentiators of cellular responses could be of interest when targeting cell lines that are resistant to therapeutic treatment, and Corning® Epic® Technology has the capability to detect these enhanced responses for endogenous receptors.

In conclusion, these data demonstrate that Corning Epic Technology can be used to investigate kinases involved in specific endogenous RTK responses in standard cell lines. Furthermore, these responses can be profiled in detail through the identification of contributing kinases involved downstream of receptor activation. Finally, this application of Corning Epic Technology can be used to broadly profile signal transduction pathways downstream of any receptor.

## Conclusions

- ▶ Endogenous RTK responses can be measured with Corning Epic Technology.
- ▶ Corning Epic Technology can be used to identify specific pathway inhibitors and potentiators of an endogenous RTK response.
- ▶ Measurement of a real-time kinetic response allows the characterization of inhibitors to determine their impact on specific temporal aspects of the response profile.
- ▶ Corning Epic Technology can be used to profile signaling pathways of an endogenous cellular response using commercially available tool compounds.

**Table 2.** Inhibitors that potentiate the EGF response at 30 or 60 minutes. Kinases with potentiator activity show an increase in EGF signal by >50% at 30 or 60 minutes.

Inhibitor	Target
<b>&gt;50% potentiation at 30 or 60 minutes</b>	
PP1	Src
PP2	Src
Aminopurvalanol A	Cdk
ZM 449829	JAK 3
ZM 447439	Aurora kinase B
IMD0354	IKK-2

## References

1. Dhomen NS, Mariadason J, Tebbutt N, Scott AM. Therapeutic targeting of the epidermal growth factor receptor in human cancer. *Crit Rev Oncog.* 17:31-50 (2012).
2. Seshacharyulu P, Ponnusamy MP, Haridas D, Jain M, Ganti AK, Batra SK. Targeting the EGFR signaling pathway in cancer therapy. *Expert Opin Ther Targets.* 16:15-31 (2012).
3. Ye F, Ferrie AM, Fontaine NH, Yuen PK. Characteristics of dynamic mass redistribution of epidermal growth factor receptor signaling in living cells measured with label-free optical biosensors. *Anal. Chem.* 77:5720-5725 (2005).



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