Measuring Luminescence of the Kinase-Glo® Luminescent Kinase Assay using the GloMax® Discover System

Promega Corporation

Materials Required

- Kinase-Glo® Luminescent Kinase Assay System (Cat.# V6711, V6712, V6713, V6714, V3771, V3772, V3773, V3774, V6071, V6072, V6073 and V6074)
- GloMax® Discover System (Cat.# GM3000)
- white, 96-well half-area assay plates (Corning Cat.# 3693)
- Nuclease-Free Water (Cat.# P1195)
- EGFR Kinase Enzyme System (Cat.# V3831; contains EGFR Kinase, Poly (4:1 Glu, Tyr) Peptide Substrate, 5X Reaction Buffer A, DTT and $MnCl_2$)

Caution: We recommend the use of gloves, lab coats and eye protection when working with these or any chemical reagents.

Protocols: *GloMax® Discover System Technical Manual* #TM397 and *Kinase-Glo® Luminescent Kinase Assay Technical Bulletin* #TB372 are available at: **www.promega.com/protocols/**

Kinases are enzymes that catalyze the transfer of a phosphate group from ATP to substrate and play an important role in cancer biology. The GloMax® Discover System in conjunction with the Kinase-Glo® Luminescent Kinase Assay provides a universal, homogeneous method to measure kinase activity by quantifying depletion of ATP in solution following a kinase reaction. The luminescent signal is correlated with the amount of ATP present and inversely correlated with the amount of kinase activity.

The Kinase-Glo® Luminescent Kinase Assays are available in three formats to monitor kinase activity at ATP concentrations of 10µM to 500µM. The Kinase-Glo® Assays are performed in a single well of a multiwell plate by adding a volume of Kinase-Glo® Reagent equal to the volume of a completed kinase reaction and measuring luminescence. ATP depletion can be monitored in a highly sensitive manner through the use of Kinase-Glo® Reagent, which uses luciferin, oxygen and ATP as substrates in a reaction that produces oxyluciferin and light. The luminescent signal is correlated with the amount of ATP present and inversely correlated with the amount of kinase activity. These assays can be performed with virtually any kinase and substrate combination and do not require radioactively labeled components. The kinase substrate can be a peptide, protein, lipid or sugar. The Kinase-Glo® Luminescent Kinase Assays easily detect known kinase inhibitors, produce excellent Z´-factor values and distinguish between ATP-competitive and ATP-noncompetitive inhibitors.

The Kinase-Glo® Luminescent Kinase Assay is made easy on the GloMax® Discover System, and the protocol comes preloaded on the instrument. The extended dynamic range and minimal well-to-well cross talk of the GloMax® Discover System allows you to easily measure signals of varying intensities on the same plate. This Application Note describes the protocol for measuring luminescence from an EGFR kinase reaction using the Kinase-Glo® Luminescent Kinase Assay and GloMax® Discover System.

Kinase-Glo® Luminescent Kinase Assay Protocol

For detailed instructions and assay notes for various assay volumes and plate formats, see the *Kinase-Glo® Luminescent Kinase Assay Technical Bulletin* #TB372. A sample procedure follows.

1. Prepare 2ml of 1X Reaction Buffer A by combining 1.6ml of Nuclease-Free Water and 400µl of 5X Reaction Buffer A (200mM Tris [pH 7.5], 100mM $MgCl_2$ and 0.5mg/ml BSA).
2. Prepare a working stock of EGFR kinase containing 32ng/µl enzyme in 1X Reaction Buffer A.
3. Prepare 2ml of a 2X ATP/Substrate mixture by combining 1.433ml of Nuclease-Free Water, 400µl of 5X Reaction Buffer A, 160µl of 1mg/ml Poly (Glu_4,Tyr_1) Substrate, 2µl of 10mM ATP, 2µl of 0.1M DTT, and 3.2µl of 2.5M $MnCl_2$.

   Note: The final ATP and Substrate concentrations will vary depending on the kinase and reaction conditions. These concentrations will need to be optimized.
4. Perform a serial twofold titration of EGFR kinase in 1X Reaction Buffer A.
5. Transfer 12.5µl of each enzyme dilution to triplicate wells of a white, half-area 96-well plate.
6. Transfer 12.5µl of 2X ATP/Substrate mix to each well. The final EGFR reaction contains 1µg of 1mg/ml Poly (Glu_4,Tyr_1) Substrate, 5µM ATP, 2mM $MnCl_2$, 50µM DTT and 1X Reaction Buffer A.
7. Shake the plate for 30 seconds, and incubate at room temperature for 1 hour.
8. Transfer 25µl of Kinase-Glo® Reagent to each well of the assay plate.
9. Shake the plate for 30 seconds, and incubate at room temperature for 10 minutes.
10. Measure luminescence (kinase activity) on the GloMax® Discover System using the Discover Kinase-Glo® protocol.

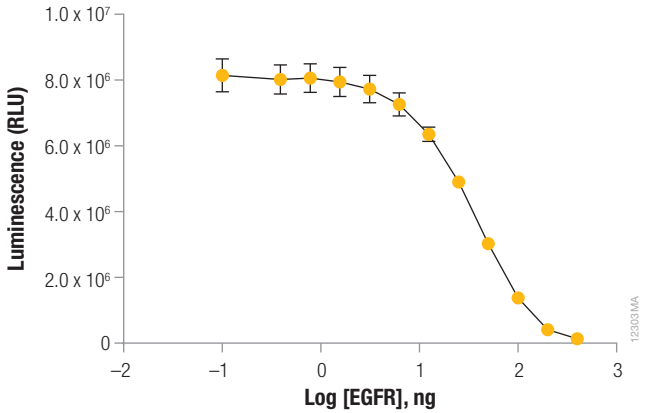

Figure 1. The luminescent signal is correlated with the amount of ATP present in the reaction and therefore inversely correlated with the amount of EGFR kinase activity. EGFR kinase was prepared in 1X Reaction Buffer A and added to a half-area 96-well plate. An equal volume of 2X ATP/Substrate mix was prepared according to Step 3 of the protocol in this document and added to each well of the plate. The final EGFR reaction contained 1µg of 1mg/ml Poly (Glu_4,Tyr_1) Substrate, 5µM ATP, 2mM $MnCl_2$, 50µM DTT and 1X Reaction Buffer A. The assay was performed as described in the *Kinase-Glo® Luminescent Kinase Assay Technical Bulletin* #TB372, and luminescence (kinase activity) was measured using the GloMax® Discover System and the Discover Kinase-Glo® protocol.

Conclusion

This Application Note demonstrates that the GloMax® Discover can measure luminescence from an EGFR kinase reaction using the Kinase-Glo® Luminescent Kinase Assay.

The GloMax® Discover System

The GloMax® Discover System offers superior sensitivity and dynamic range and limited well-to-well cross talk. The instrument was developed and optimized with Promega cell and gene reporter assays and may be integrated into low- and medium-throughput automation workflows. The GloMax® Discover System allows flexible use of filters to measure fluorescence intensity, filtered luminescence, BRET, FRET and UV-visible absorbance for a wide variety of laboratory applications. The instrument is operated by an integrated Tablet PC, which provides quick and easy navigation through the control options. Exporting your results is made seamless with a variety of options, including exporting data to your local network.

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