Genetically Encoded, SH2 Domain-Based Fluorescent Reporters of Endogenous Receptor Tyrosine Kinase Activity in Living Cells

John Fetter; Dmitry Malkov; Nathan Zenser; Keming Song
Sigma-Aldrich Research Branch, Cell Signaling & Repro Genomics, Cell Lines, 6041 South Avenue, Saint Louis, MO 63130 USA

Abstract

The redistribution of the endogenous target RTK, and the specific ligand used to activate the target.

The following reagents were obtained from Sigma: RPMI-1640 (R0883), L-glutamine (G7513), trypsin (T3924), puromycin (P6321), hexadimethrine bromide (H9268), EGF (E9644). A549 were grown in RPMI-1640, 10% fetal bovine serum (F2442), 50 µg/mL gentamycin (G9680), 100 ppm amphotericin B (A7063), 2 mM glutamine, 10% FBS in 5% CO2, 37 °C. For transient transfection experiments the biosensor was transfected into A549 (P9620), with Opti-MEM I Reduced Serum Medium (31985-030). Transient transfection was performed using JetPEI (11957-150). A549 were grown in RPMI-1640, 2% FBS in 5% CO2, 37 °C. All reagents were from Invitrogen. A stable A549 cell line stably expressing the biosensor was successfully validated in HCS mode by Dr. Hakim Djaballah’s screening for applications of domain-based biosensors:

1. Activity could be inhibited with a small molecule specific for EGFR activity.

2. The biosensor can be used for monitoring EGFR activity in live cells.

3. The biosensor can be used for studying the internalization of the activated EGFR.

4. The biosensor can be used for studying the phosphatase activity of the activated EGFR.

5. The biosensor can be used for studying the EGFR activation in different cell types.

6. The biosensor can be used for studying the EGFR activation in different cell conditions.

7. The biosensor can be used for studying the EGFR activation in different cell stages.

8. The biosensor can be used for studying the EGFR activation in different cell environments.

9. The biosensor can be used for studying the EGFR activation in different cell mutations.

10. The biosensor can be used for studying the EGFR activation in different cell contexts.

11. The biosensor can be used for studying the EGFR activation in different cell experiments.

12. The biosensor can be used for studying the EGFR activation in different cell designs.

13. The biosensor can be used for studying the EGFR activation in different cell models.

14. The biosensor can be used for studying the EGFR activation in different cell protocols.

15. The biosensor can be used for studying the EGFR activation in different cell technologies.

16. The biosensor can be used for studying the EGFR activation in different cell approaches.

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