NORAK BIOSCIENCES INC.

Transfluor® Technology

Universal
GPCR Assay for Known & Orphan Receptors & All Ligand Types

Highly Reproducible

Very Sensitive

Simple & Low Cost

High IQ™
Image Quantification Assay

High Throughput

High Content

Norak Transfluor® Publications

1. Robert Oakley, Christine Hudson, Rachael Cruickshank, Diane Meyers, Richard Payne, Jr., Shay Rhem, and Carson Loomis. (2002). The Cellular Distribution of Fluorescently Labeled Arrestins Provides a Robust, Sensitive, and Universal Assay for Screening G Protein-Coupled Receptors. ASSAY and Drug Development Technologies 1(1): 21-30.
2. Christine Hudson, Robert Oakley, Rachael Cruickshank, Shay Rhem, and Carson Loomis. (2002). Automation and Validation of the Transfluor® Technology: a Universal Screening Assay for G Protein-coupled Receptors. Proceedings of SPIE. Volume 4626: 548-55.

GPCR Desensitization Mechanism Publications from Scientific Founders

1. Ahn S, Nelson CD, et al. (2003) Desensitization, internalization, and signaling functions of beta-arrestins demonstrated by RNA interference. Proc Natl Acad Sci U S A 100(4):1740-4
2. Attramadal, H., J. L. Arriza, et al. (1992). Beta-arrestin2, a novel member of the arrestin/beta-arrestin gene family. J Biol Chem 267(25): 17882-17890.
3. **Barak, L. S., S. S. Ferguson, et al. (1997). A beta-arrestin/green fluorescent protein biosensor for detecting G protein-coupled receptor activation. J Biol Chem 272(44): 27497-500.**
4. **Barak, L. S., S. S. Ferguson, et al. (1997). Internal trafficking and surface mobility of a functionally intact beta2-adrenergic receptor-green fluorescent protein conjugate. Mol Pharmacol 51(2): 177-84.**
5. Barak, L. S., L. Menard, et al. (1995). The conserved seven-transmembrane sequence NP(2,3Y of the G-protein-coupled receptor superfamily regulates multiple properties of the beta 2-adrenergic receptor. Biochemistry 34(47): 15407-14.
6. **Barak, L. S., R. H. Oakley, et al. (2001). Constitutive arrestin-mediated desensitization of a human vasopressin receptor mutant associated with nephrogenic diabetes insipidus. Proc Natl Acad Sci U S A 98: 93-98.**
7. Barak, L. S., M. Tiberi, et al. (1994). A highly conserved tyrosine residue in G protein-coupled receptors is required for agonist-mediated beta 2-adrenergic receptor sequestration. J Biol Chem 269(4): 2790-2795.
8. **Barak, L. S., K. Warabi, et al. (1999). Real-time visualization of the cellular redistribution of G protein-coupled receptor kinase 2 and beta-arrestin 2 during homologous desensitization of the substance P receptor. J Biol Chem 274(11): 7565-9.**

9. Bektas, M, Barak, L.S., et al. (2003). The G Protein-Coupled Receptor GPR4 Suppresses ERK Activation in a Ligand-Independent Manner. Biochemistry 42, 12181-12191.
10. Benovic, J. L., F. J. Mayor, et al. (1987). Purification and characterization of the beta-adrenergic receptor kinase. J Biol Chem 262(19): 9026-9032.
11. Benovic, J. L., J. J. Onorato, et al. (1991). Cloning, expression, and chromosomal localization of beta-adrenergic receptor kinase 2. A new member of the receptor kinase family. J Biol Chem 266(23): 14939-14946.
12. Benovic, J. L., J. W. Regan, et al. (1987). Agonist-dependent phosphorylation of the alpha 2-adrenergic receptor by the beta-adrenergic receptor kinase. J Biol Chem 262(36): 17251-17253.
13. Benovic, J. L., C. Staniszewski, et al. (1988). beta-Adrenergic receptor kinase. Activity of partial agonists for stimulation of adenylate cyclase correlates with ability to promote receptor phosphorylation. J Biol Chem 263(8): 3893-7.
14. Benovic, J. L., W. C. Stone, et al. (1989). Inhibition of the beta-adrenergic receptor kinase by polyanions. J Biol Chem 264(12): 6707-6710.
15. Blitzer, R. D., G. Omri, et al. (1993). Coupling of the expressed alpha 1B-adrenergic receptor to the phospholipase C pathway in Xenopus oocytes. The role of Go. J Biol Chem 268(10): 7532-7537.
16. Bohn, L. M., R. R. Gainetdinov, et al. (2000). Mu-opioid receptor desensitization by beta-arrestin-2 determines morphine tolerance but not dependence. Nature 408(6813): 720-3.
17. Bohn, L. M., R. J. Lefkowitz, et al. (1999). Enhanced morphine analgesia in mice lacking beta-arrestin 2. Science 286(5449): 2495-8.
18. Bohn, L. M., F. Xu, et al. (2000). Potentiated opioid analgesia in norepinephrine transporter knock-out mice. J Neurosci 20(24): 9040-5.
19. Bouvier, M., S. Collins, et al. (1989). Two distinct pathways for cAMP-mediated down-regulation of the beta 2- adrenergic receptor. Phosphorylation of the receptor and regulation of its mRNA level. J Biol Chem 264(28): 16786-16792.
20. Carman, C. V., L. S. Barak, et al. (2000). Mutational Analysis of Gßg and Phospholipid Interaction with G Protein-coupled Receptor Kinase 2. J Biol Chem 275(14): 10443-10452.
21. Cerione, R. A., J. Codina, et al. (1985). Transducin and the inhibitory nucleotide regulatory protein inhibit the stimulatory nucleotide regulatory protein mediated stimulation of adenylate cyclase in phospholipid vesicle systems. Biochemistry 24(17): 4499-4503.
22. Cerione, R. A., P. Gierschik, et al. (1987). Functional differences in the beta gamma complexes of transducin and the inhibitory guanine nucleotide regulatory protein. Biochemistry 26(5): 1485-91.

23. Cerione, R. A., C. Staniszewski, et al. (1986). Mechanism of guanine nucleotide regulatory protein-mediated inhibition of adenylate cyclase. Studies with isolated subunits of transducin in a reconstituted system. J Biol Chem 261(20): 9514-20.
24. Claing A, Laporte SA, et al. (2002) Endocytosis of G protein-coupled receptors: roles of G protein-coupled receptor kinases and beta-arrestin proteins. Prog Neurobiol 66(2):61-79
25. Daaka, Y., L. M. Luttrell, et al. (1998). Essential role for G protein-coupled receptor endocytosis in the activation of mitogen-activated protein kinase. J Biol Chem 273(2): 685-8.
26. Daaka, Y., J. A. Pitcher, et al. (1997). Receptor and G betagamma isoform-specific interactions with G protein-coupled receptor kinases. Proc Natl Acad Sci U S A 94(6): 2180-5.
27. Feng, X., J. Zhang, et al. (1998). Visualization of Dynamic Trafficking of a Protein Kinase C ßII/Green Fluorescent Protein Conjugate Reveals Differences in G Protein-coupled Receptor Activation and Desensitization. J Biol Chem 276(17): 10755-10762.
28. Ferguson, S. S., L. S. Barak, et al. (1996). G-protein-coupled receptor regulation: role of G-protein-coupled receptor kinases and arrestins. Can J Physiol Pharmacol 74(10): 1095-110.
29. Ferguson, S. S. and M. G. Caron (1998). G protein-coupled receptor adaptation mechanisms. Semin Cell Dev Biol 9(2): 119-27.
30. **Ferguson, S. S., W. E. Downey, 3rd, et al. (1996). Role of beta-arrestin in mediating agonist-promoted G protein-coupled receptor internalization. Science 271(5247): 363-6.**
31. Ferguson, S. S., L. Menard, et al. (1995). Role of Phosphorylation in Agonist-promoted -Adrenergic Receptor Sequestration, RESCUE OF A SEQUESTRATION-DEFECTIVE MUTANT RECEPTOR BY ARK1. J Biol Chem 270(42): 24782-24789.
32. **Ferguson, S. S., J. Zhang, et al. (1998). Molecular mechanisms of G protein-coupled receptor desensitization and resensitization. Life Sci 62(17-18): 1561-5.**
33. **Ferguson, S. S., J. Zhang, et al. (1998). Role of beta-arrestins in the intracellular trafficking of G-protein-coupled receptors. Adv Pharmacol 42: 420-4.**
34. Freedman, N. J., A. S. Ament, et al. (1997). Phosphorylation and desensitization of human endothelin A and B receptors. Evidence for G protein-coupled receptor kinase specificity. J Biol Chem 272(28): 17734-43.
35. **Freedman, N. J. and R. J. Lefkowitz (1996). Desensitization of G protein-coupled receptors. Recent Prog Horm Res 51: 319-51.**

36. Freedman, N. J., S. B. Liggett, et al. (1995). Phosphorylation and Desensitization of the Human -Adrenergic Receptor - INVOLVEMENT OF G PROTEIN-COUPLED RECEPTOR KINASES AND cAMP-DEPENDENT PROTEIN KINASE. J Biol Chem 270(30): 17953-17961.
37. Gainetdinov, R. R., L. M. Bohn, et al. (1999). Muscarinic supersensitivity and impaired receptor desensitization in G protein-coupled receptor kinase 5-deficient mice. Neuron 24(4): 1029-36.
38. Gainetdinov, R. R. and M. G. Caron (1999). Genetic models in pharmacology: present status and future. Pharmacol Res 39(6): 403-4.
39. Hall, R. A., R. F. Spurney, et al. (1999). G protein-coupled receptor kinase 6A phosphorylates the Na(+)/H(+) exchanger regulatory factor via a PDZ domain-mediated interaction. J Biol Chem 274(34): 24328-34.
40. Hausdorff, W. P., M. Bouvier, et al. (1989). Phosphorylation sites on two domains of the beta 2-adrenergic receptor are involved in distinct pathways of receptor desensitization. J Biol Chem 264(21): 12657-12665.
41. Inglese, J., N. J. Freedman, et al. (1993). Structure and mechanism of the G protein-coupled receptor kinases. J Biol Chem 268(32): 23735-8.
42. Inglese, J., J. F. Glickman, et al. (1992). Isoprenylation of a protein kinase. Requirement of farnesylation/alpha- carboxyl methylation for full enzymatic activity of rhodopsin kinase. J Biol Chem 267(3): 1422-1425.
43. Kim, K. M., K. J. Valenzano, et al. (2001). Differential Regulation of the Dopamine D2 and D3 Receptors by G Protein-coupled Receptor Kinases and beta-Arrestins. J Biol Chem 276(40): 37409-14.
44. Koch, W. J., J. Inglese, et al. (1993). The binding site for the beta gamma subunits of heterotrimeric G proteins on the beta-adrenergic receptor kinase. J Biol Chem 268(11): 8256-60.
45. Kohout TA, Lefkowitz RJ. (2003) Regulation of G protein-coupled receptor kinases and arrestins during receptor desensitization. Mol Pharmacol 63(1):9-18
46. Kohout, T. A., F. S. Lin, et al. (2001). beta-Arrestin 1 and 2 differentially regulate heptahelical receptor signaling and trafficking. Proc Natl Acad Sci U S A 98(4): 1601-6.
47. Krupnick, J. G. and J. L. Benovic (1998). The role of receptor kinases and arrestins in G protein-coupled receptor regulation. Annu. Rev. Pharmacol. Toxicol. 38: 289-319.
48. Kwatra, M. M., D. A. Schwinn, et al. (1993). The substance P receptor, which couples to Gq/11, is a substrate of beta-adrenergic receptor kinase 1 and 2. J Biol Chem 268(13): 9161-9164.
49. Laporte SA, Miller WE, et al. (2002) beta-Arrestin/AP-2 interaction in G protein-coupled receptor internalization: identification of a beta-arrestin binging site in beta 2-adaptin. J Biol Chem 277(11):9247-54

50. **Laporte, S. A., R. H. Oakley, et al. (2000). The interaction of beta-arrestin with the AP-2 adaptor is required for the clustering of beta 2-adrenergic receptor into clathrin-coated pits. J Biol Chem 275(30): 23120-6.**
51. **Laporte, S. A., R. H. Oakley, et al. (1999). The beta2-adrenergic receptor/betaarrestin complex recruits the clathrin adaptor AP-2 during endocytosis. Proc Natl Acad Sci U S A 96(7): 3712-7.**
52. Leeb-Lundberg, L. M., S. Cotecchia, et al. (1987). Regulation of adrenergic receptor function by phosphorylation. I. Agonist-promoted desensitization and phosphorylation of alpha 1-adrenergic receptors coupled to inositol phospholipid metabolism in DDT1 MF-2 smooth muscle cells. J Biol Chem 262(7): 3098-105.
53. Liggett, S. B., J. Ostrowski, et al. (1992). Sites in the third intracellular loop of the alpha 2A-adrenergic receptor confer short term agonist-promoted desensitization. Evidence for a receptor kinase-mediated mechanism. J Biol Chem 267(7): 4740-4746.
54. Lohse, M. J., S. Andexinger, et al. (1992). Receptor-specific desensitization with purified proteins. Kinase dependence and receptor specificity of beta-arrestin and arrestin in the beta 2-adrenergic receptor and rhodopsin systems. J Biol Chem 267(12): 8558-8564.
55. Lohse, M. J., J. L. Benovic, et al. (1990). Multiple pathways of rapid beta 2-adrenergic receptor desensitization. Delineation with specific inhibitors. J Biol Chem 265(6): 3202-3211.
56. Lohse, M. J., J. L. Benovic, et al. (1990). beta-Arrestin: a protein that regulates beta-adrenergic receptor function. Science 248(4962): 1547-50.
57. Luttrell, L. M., S. S. Ferguson, et al. (1999). Beta-arrestin-dependent formation of beta2 adrenergic receptor-Src protein kinase complexes. Science 283(5402): 650-1.
58. Luttrell LM, Lefkowitz RJ. (2002) The role of beta-arrestins in the termination and transduction of G-protein-coupled receptor signals. J Cell Sci 115(Pt 3):455-65
59. Macrae, A. D., R. T. Premont, et al. (1996). Cloning, characterization, and chromosomal localization of rec1.3, a member of the G-protein-coupled receptor family highly expressed in brain. Brain Res Mol Brain Res 42(2): 245-54.
60. Mayor, F. J., J. L. Benovic, et al. (1987). Somatostatin induces translocation of the beta-adrenergic receptor kinase and desensitizes somatostatin receptors in S49 lymphoma cells. J Biol Chem 262(14): 6468-6471.
61. McDonald, P. H., C. W. Chow, et al. (2000). Beta-arrestin 2: a receptor-regulated MAPK scaffold for the activation of JNK3. Science 290(5496): 1574-7.
62. McDonald, P. H. and R. J. Lefkowitz (2001). betaArrestins. New roles in regulating heptahelical receptors' functions. Cell Signal 13(10): 683-9.

63. Menard, L., S. S. Ferguson, et al. (1997). Synergistic regulation of beta2-adrenergic receptor sequestration: intracellular complement of beta-adrenergic receptor kinase and beta-arrestin determine kinetics of internalization. Mol Pharmacol 51(5): 800-8.
64. Mhaouty-Kodja S, L. S. Barak, et al. (1999). Constitutively active alpha-1b adrenergic receptor mutants display different phosphorylation and internalization features. Mol Pharmacol 55(2): 339-47.
65. Miller, W. E. and R. J. Lefkowitz (2001). Expanding roles for beta-arrestins as scaffolds and adapters in GPCR signaling and trafficking. Curr Opin Cell Biol 13(2): 139-45.
66. Naga Prasad, S. V., L. S. Barak, et al. (2001). Agonist-dependent recruitment of phosphoinositide 3-kinase to the membrane by beta-adrenergic receptor kinase 1. A role in receptor sequestration. J Biol Chem 276(22): 18953-9.
67. Oakley, R. H., S. A. Laporte, et al. (1999). Association of beta-arrestin with G protein-coupled receptors during clathrin-mediated endocytosis dictates the profile of receptor resensitization. J Biol Chem 274(45): 32248-57.
68. Oakley, R. H., S. A. Laporte, et al. (2001). Molecular determinants underlying the formation of stable intracellular G protein-coupled receptor-beta-arrestin complexes after receptor endocytosis*. J Biol Chem 276(22): 19452-60.
69. Oakley, R. H., S. A. Laporte, et al. (2000). Differential affinities of visual arrestin, beta arrestin1, and beta arrestin2 for G protein-coupled receptors delineate two major classes of receptors. J Biol Chem 275(22): 17201-10.
70. Oppermann, M., N. J. Freedman, et al. (1996). Phosphorylation of the type 1A angiotensin II receptor by G protein-coupled receptor kinases and protein kinase C. J Biol Chem 271(22): 13266-72.
71. Pei, G., P. Samama, et al. (1994). A constitutively active mutant beta 2-adrenergic receptor is constitutively desensitized and phosphorylated. Proc Natl Acad Sci U S A 91(7): 2699-702.
72. Peppel, K., I. Boekhoff, et al. (1997). G Protein-coupled Receptor Kinase 3 (GRK3) Gene Disruption Leads to Loss of Odorant Receptor Desensitization. J Biol Chem 272(41): 25425-25428.
73. Pierce, K. L. and R. J. Lefkowitz (2001). Classical and New Roles of beta-arrestins in the Regulation of G-protein-coupled Receptors. Nat Rev Neurosci 2(10): 727-33.
74. Pierce, K. L., L. M. Luttrell, et al. (2001). New mechanisms in heptahelical receptor signaling to mitogen activated protein kinase cascades. Oncogene 20(13): 1532-9.
75. Pierce, K. L., S. Maudsley, et al. (2000). Role of endocytosis in the activation of the extracellular signal-regulated kinase cascade by sequestering and

nonsequestering G protein-coupled receptors. Proc Natl Acad Sci U S A 97(4): 1489-94.

76. Pippig, S., S. Andexinger, et al. (1993). Overexpression of beta-arrestin and beta-adrenergic receptor kinase augment desensitization of beta 2-adrenergic receptors. J Biol Chem 268(5): 3201-3208.
77. Pitcher, J. A., Z. L. Fredericks, et al. (1996). Phosphatidylinositol 4,5-bisphosphate (PIP2)-enhanced G protein-coupled receptor kinase (GRK) activity. Location, structure, and regulation of the PIP2 binding site distinguishes the GRK subfamilies. J Biol Chem 271(40): 24907-13.
78. Pitcher, J. A., R. A. Hall, et al. (1998). The G protein-coupled receptor kinase 2 is a microtubule-associated protein kinase that phosphorylates tubulin. J Biol Chem 273(20): 12316-24.
79. Pitcher, J. A., J. J. Tesmer, et al. (1999). Feedback inhibition of G protein-coupled receptor kinase 2 (GRK2) activity by extracellular signal-regulated kinases. J Biol Chem 274(49): 34531-4.
80. Premont, R. T., J. Inglese, et al. (1995). Protein kinases that phosphorylate activated G protein-coupled receptors. The FASEB Journal 9: 175-182.
81. Rockman, H. A., D. J. Choi, et al. (1998). Control of Myocardial Contractive Function by the Level of ß-Adrenergic Receptor Kinase ! in Gene-targeted Mice. J Biol Chem 273(29): 18180-18184.
82. Samama, P., S. Cotecchia, et al. (1993). A mutation-induced activated state of the beta 2-adrenergic receptor. Extending the ternary complex model. J Biol Chem 268(7): 4625-36.
83. Shenoy SK, Lefkowitz RJ. (2003) Trafficking Patterns of beta -Arrestin and G Protein-coupled Receptors Determined by the Kinetics of beta -Arrestin Deubiquitination. J Biol Chem 278(16):14498-506
84. Shenoy, S. K., P. H. McDonald, et al. (2001). Regulation of Receptor Fate by Ubiquitination of Activated {beta}2-Adrenergic Receptor and {beta}-Arrestin. Science.
85. Shetzline, M. A., R. T. Premont, et al. (1998). A Role for Receptor Kinases in the Regulation of Class II G Protein-coupled Receptors. J Biol Chem 273(12): 6756-6762.
86. Stadel, J. M., P. Nambi, et al. (1983). Catecholamine-induced desensitization of turkey erythrocyte adenylate cyclase is associated with phosphorylation of the beta-adrenergic receptor. Proc Natl Acad Sci U S A 80(11): 3173-7.
87. Strasser, R. H., J. L. Benovic, et al. (1986). Beta-agonist- and prostaglandin E1-induced translocation of the beta-adrenergic receptor kinase: evidence that the kinase may act on multiple adenylate cyclase-coupled receptors. Proc Natl Acad Sci U S A 83(17): 6362-6.

88. Strasser, R. H. and R. J. Lefkowitz (1985). Homologous desensitization of beta-adrenergic receptor coupled adenylate cyclase. Resensitization by polyethylene glycol treatment. J Biol Chem 260(8): 4561-4.
89. Strulovici, B., J. M. Stadel, et al. (1983). Functional integrity of desensitized beta-adrenergic receptors. J Biol Chem 258(10): 6410-4.
90. Tiberi, M., S. R. Nash, et al. (1996). Differential Regulation of Dopamine D1A Receptor Responsiveness by Various G Protein-coupled Receptor Kinases. J Biol Chem 271(7): 3771-3778.
91. Walker, J. K., K. Peppel, et al. (1999). Altered airway and cardiac responses in mice lacking G protein-coupled receptor kinase 3. Am J Physiol 276(4 Pt 2): R1214-21.
92. Walker, J. K., R. T. Premont, et al. (1999). Properties of secretin receptor internalization differ from those of the beta(2)-adrenergic receptor. J Biol Chem 274(44): 31515-23.
93. Zhang, J., L. S. Barak, et al. (1999). Cellular trafficking of G protein-coupled receptor/beta-arrestin endocytic complexes. J Biol Chem 274(16): 10999-1006.
94. Zhang, J., L. S. Barak, et al. (1997). A central role for beta-arrestins and clathrin-coated vesicle-mediated endocytosis in beta2-adrenergic receptor resensitization. Differential regulation of receptor resensitization in two distinct cell types. J Biol Chem 272(43): 27005-14.
95. Zhang, J., S. S. Ferguson, et al. (1996). Dynamin and beta-arrestin reveal distinct mechanisms for G protein-coupled receptor internalization. J Biol Chem 271(31): 18302-5.
96. Zhang, J., S. S. Ferguson, et al. (1999). Agonist-specific regulation of delta-opioid receptor trafficking by G protein-coupled receptor kinase and beta-arrestin. J Recept Signal Transfuct Res 19(1-4): 301-13.