



Data Sheet

GPCRProfiler[®] Services

Evolving with You to Advance GPCR Drug Discovery

RELIABILITY, FLEXIBILITY, & SPEED

GPCRs, or seven transmembrane receptors, continue to be a major focus for drug discovery. And, finding a GPCR service that meets all your needs can be challenging. These days, you're faced with increasing pressure to develop drugs in a shorter time frame while passing the FDA's scrutiny for efficacy and safety. That means screens must be implemented rapidly to identify lead compounds. In turn, these compounds must be triaged quickly and optimized at early stages to avoid wasting time and resources in developing compounds that turn out to be inherently flawed. Fortunately, Millipore can help you at all stages of this process—more cost-effectively than you might expect.

PROBLEM SOLVED

Millipore's GPCRProfiler Service was the first provider of cell-based functional assays to take advantage of this rich data readout that includes both agonist and antagonist data. GPCRProfiler Service can help in any stage of compound development—from hit identification to compound selectivity profiling. With more than 150 GPCRs, Millipore can jumpstart your primary screening project, help in structure activity relationship (SAR) studies to optimize lead compounds and identify potential off-target interactions that may lead to drug development-stopping liabilities.

Affordable
Full Panel
& Safety
Panels



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Why Functional Assays?

Functional assays provide more relevant information than affinity-based assays, such as radioligand assays. Like affinity assays, functional assays can detect whether a compound interacts with a particular receptor. However, functional assays enable you to know much more. They enable you to:

- Determine whether a compound inhibits or activates a given receptor and with what potency and efficacy.
- Identify more relevant interactions. Often agonists, including native ligands such as acetylcholine, elicit a functional response at much lower concentration than would be suggested by their affinities. Therefore, a potentially dangerous off-target interaction of an agonist might be missed by employing an affinity-based screen.
- Gain more information for SAR studies. Affinity, intrinsic activity, efficacy and cooperatively are independent properties of a chemical. As a result, SAR driven by binding studies alone may be misleading. For example, development of compounds with higher affinity does not guarantee that the compounds will be functionally more potent or efficacious. Just as important, subtle chemical substitutions can dramatically alter the functional activity of a compound. For example, a substitution could easily transform a chemical analog from an antagonist to an agonist or vice versa.

- Quickly identify and determine the functionality of allosteric compounds. Allosteric compounds bind to a site that is distinct to orthosteric radioligand binding sites. Therefore, allosteric compounds are difficult to detect via traditional equilibrium competition binding.
- Ensure native agonist ligands are used as probes. Affinity binding assays typically use high-affinity antagonists/inverse agonists as radioligands. As drugs are developed to modulate interactions of native agonists, not synthetic molecules, radioligand binding studies may give misleading results for allosteric modulators whose activities are dependent on the orthosteric ligand. In this case, the most important orthosteric probe is the native ligand.

Why Profile for Selectivity?

Once hits are identified and confirmed for activity at the desired target, the next monumental task is to choose lead compounds and optimize their properties. Perhaps somewhat underappreciated during this phase of discovery is the identification of compound selectivity. If off-target activities are identified, it may be necessary to remove hazardous interactions or potentially optimize beneficial off-target activities.

- Many GPCRs naturally share the same ligand despite having divergent sequence.

- Similarly, many compounds and drugs are known to non-selectively interact within and even outside the GPCR family. Some compounds, such as atypical antipsychotics, have quite complex pharmacological profiles which can contribute both positively and negatively to the clinical profile of the drug [Figure 1]. In other cases, compounds initially thought to be "selective" are later rejected upon screening against a larger spectrum of targets.
- In some cases, retrospective profiling of marketed drugs can either shed light on the drug's therapeutic or side-effect mechanism of action (MOA) or reveal unknown interactions that could suggest new indications for an approved drug.
- It's important to understand how the activities of metabolites compare with that of the parent compound and what possible liabilities or benefits exist due to differences in functional selectivity.

we have extensive experience providing services for multiple target classes including kinases (**KinaseProfiler™** services), phosphatases (**PhosphataseProfiler™** services) and ion channels (**IonChannelProfiler™** services).

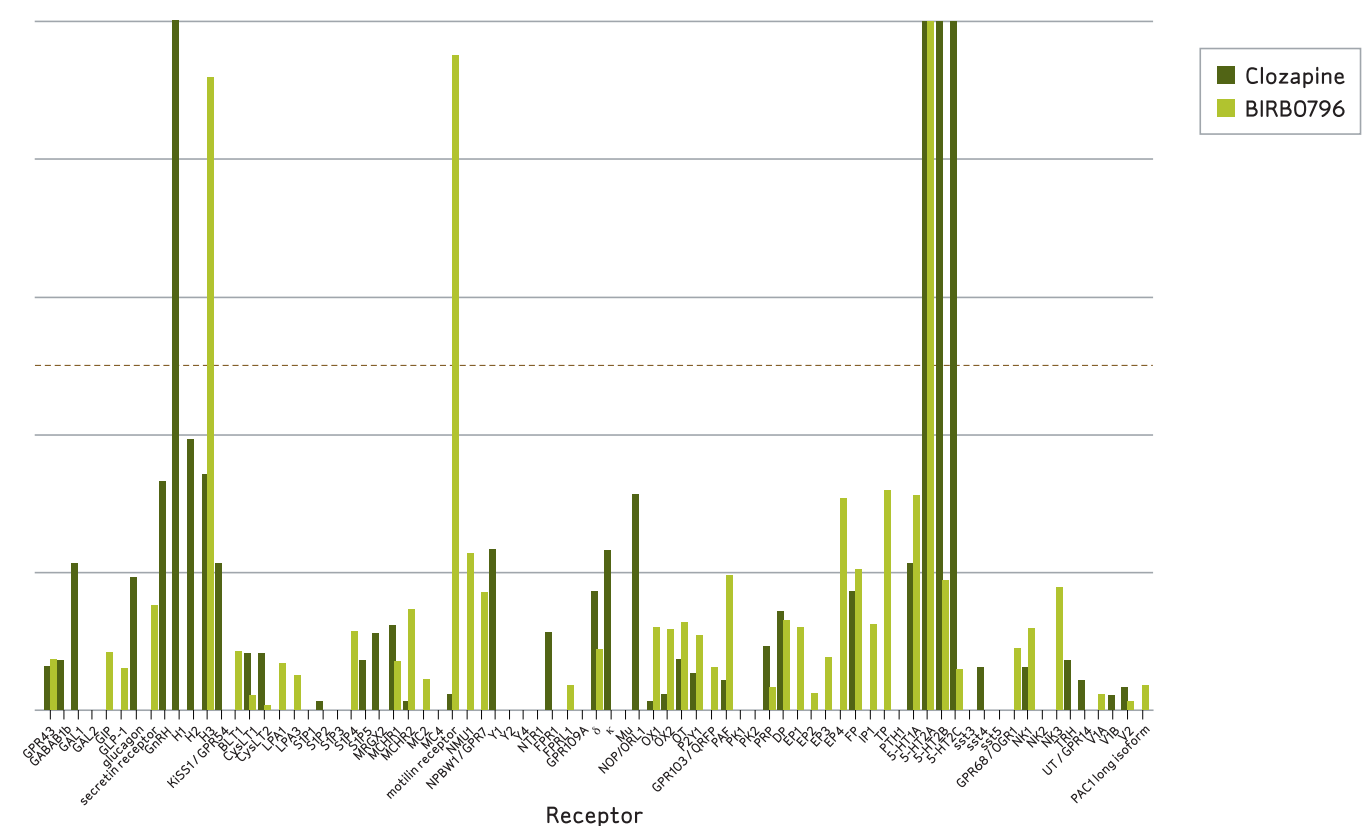
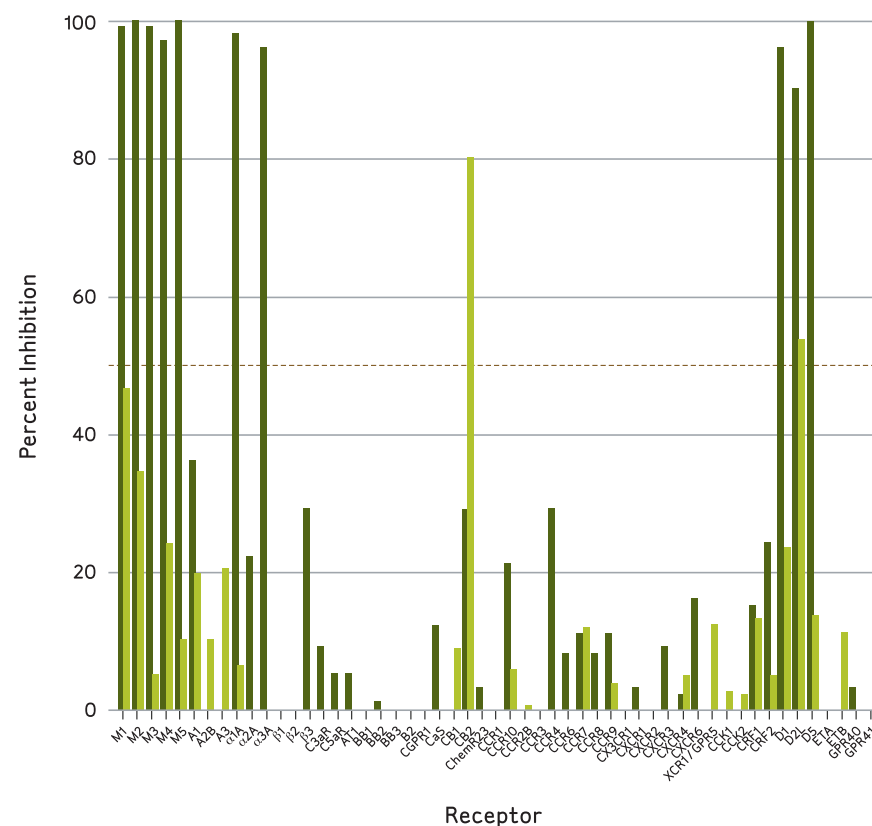
In fact, we develop and routinely perform nearly 200 different cell-based assays each week. And because we view each of our **GPCRProfiler** clients as partners in drug discovery, our GPCR experts will work with you to develop the best project plan to meet your goals.

GPCRProfiler Service Highlights:

- Cell-based functional assay are available for more than 150 GPCRs, covering more than 57 different ligand families, including an extensive collection of chemokine, lysophospholipid and biogenic amine family members.
- Dual mode screening (agonist and antagonist) in a single well means you won't miss the functionality of analogs at the directed target or against off-target receptors [Figure 2, 3 and 4].
- Our assays are performed using Millipore's stable ChemiScreen™ GPCR cell lines that are validated to work in the presence of up to 0.5% DMSO to minimize vehicle response and maximize result consistency.
- We offer a single platform using an industry standard Ca²⁺ flux assay, which enables buffer, incubation time, readout and data analysis to be consistent across receptors [see Figure 5, page 5].

Figure 1. Full Panel Profiling Reveals Interesting Off-target Hits for GPCR and Non-GPCR Directed Compounds.

Clozapine, a marketed atypical antipsychotic, and BIRB0796, a compound developed as a SAPK2a/2b kinase inhibitor, were profiled at 10 μM for agonist and antagonist activity against a large GPCR panel. Agonist activity was not detected for either compound. Percent inhibition is shown for the antagonist screen with the dotted line indicating 50 percent inhibition. BIRB0796 was not tested against CXCR6, MC4 and UT. Clozapine was not tested against BB3, CCR3, CX3CR1, XCR1, CCK1, ETB, GPR41, GAL2, GIP, GLP-1, secretin receptor, S1P1, MC2, GPR7, Y4, GPR109A, Mu, GPR103, PK2, EP2, IP1, sst5, GPR68 and NK3.



- Standard assays can be performed in either 96 or 384-plate format on a FLIPR^{TETRA}® with fully integrated and automated compound and plate handling.
- When your needs go beyond our standard service, we can easily tailor a custom project to collect the data that you need using our FlexLab GPCR services.
- GPCRProfiler's disease and safety panels developed by our GPCR experts can help direct your projects.
- We provide quick turnaround times of 1-3 weeks depending on project types:
 - Data for ongoing SAR driven projects are typically delivered in approximately one week.
 - Fixed schedule safety and full panel screens are typically delivered in approximately two weeks.
- We offer competitive prices without annual commitments.

Figure 3. How is Screening Data Generated by GPCRProfiler Service?

GPCRProfiler service performs two general types of projects: screens and dose response. Screens provide a Yes/No answer to interaction with the data provided as percent activation or inhibition. Shown in panels A and B, was a screen performed in a 96-well format (same controls are also included in 384-well assays) looking for compound activity against α_{1A} receptor (Cat. No. HTS087C). Wells shown in panels A and B are identical with panel A being the 1st addition (test for agonist activity) and panel B being the 2nd addition (test for antagonist activity). Various test compounds (blue, cyan and orange) and a control antagonist, WB 4101, (purple) are compared to negative vehicle control (green wells) and positive control agonist, epinephrine, (yellow wells) at either $[E_{max}]$ (panel A) or $[EC_{80}]$ (panel B) to establish percent activation or inhibition, respectively.

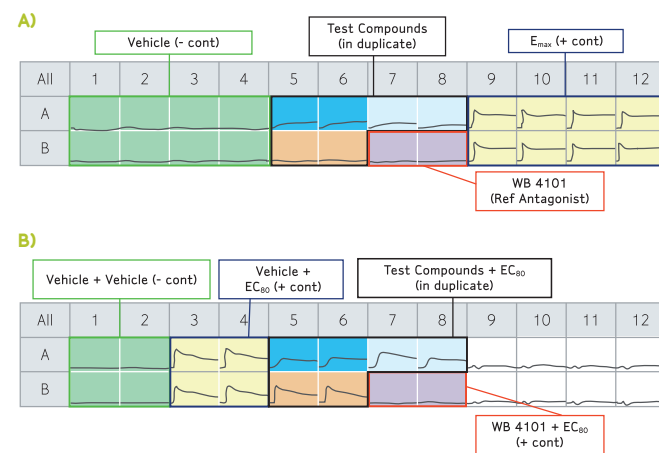


Figure 2. GPCRProfiler Detects Agonist and Antagonist Activity in a Single Well.

Millipore uses Ca^{2+} flux assays on the FLIPR^{TETRA}® platform to obtain your data. Experiments are designed to detect both agonist and antagonist activities that a compound may possess. After a baseline read, compounds are added to cells expressing a GPCR at 10 seconds to detect agonist activity. After an additional 10 minutes of incubation the cells are then challenged with an EC_{80} concentration of a reference agonist to detect whether the compound behaves as an antagonist. Examples of kinetic traces obtained from M2 Acetylcholine muscarinic receptor cell line (Cat. No. HTS115C) treated with A) vehicle control (1st and 2nd addition) - **no response**; B) E_{max} of reference agonist, 10 μM acetylcholine (1st addition) - **agonist response** detected; C) vehicle (1st addition) and reference agonist, acetylcholine, at an EC_{80} concentration (2nd addition) - **no agonist or antagonism**; and D) reference antagonist, atropine, (1st addition) and, acetylcholine, at an EC_{80} concentration (2nd addition) - **antagonist response** (i.e., blunting of the 2nd addition response see in panel C).

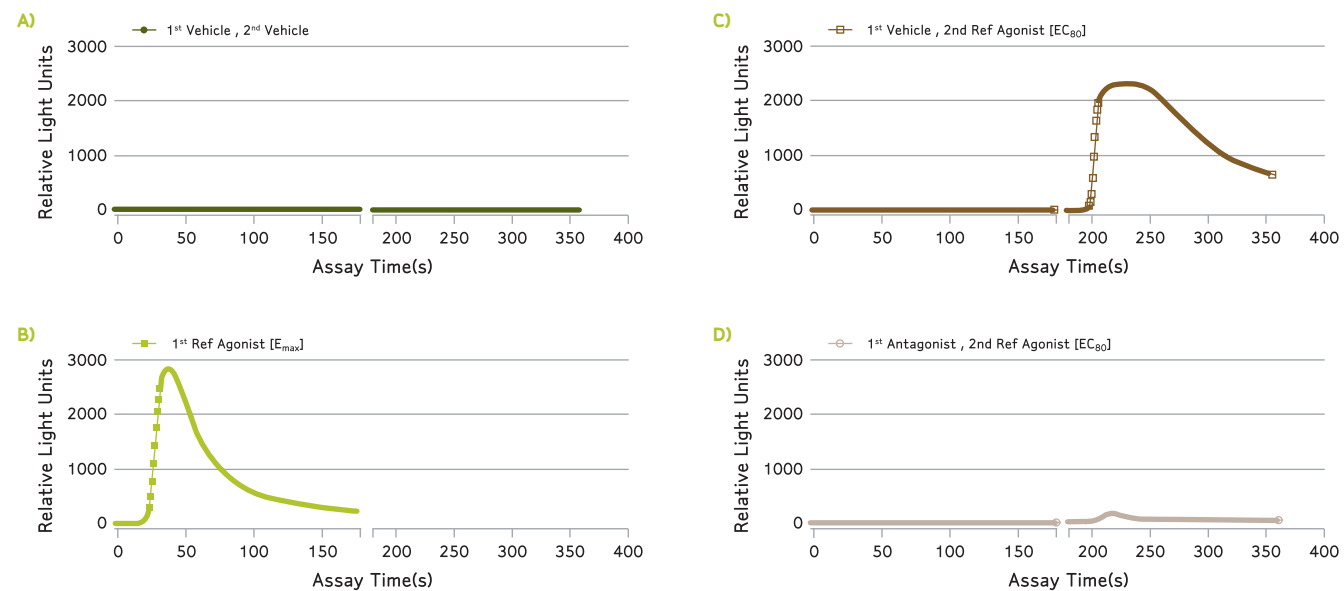


Figure 4. GPCRProfiler's Dose Response Service Drives SAR and Identifies a Compound's Window of Selectivity Between Receptors.

Dose response studies demonstrate how potent and efficacious compounds are at one or more receptors. Standard dose response experiments were performed to confirm hits from a broad screen of the marketed drugs A) clozapine (atypical antipsychotic) and B) oxymetazoline (nasal decongestant). Clozapine demonstrated non-selectivity for biogenic amine receptors which contributes to its clinical effectiveness, but also a host of side-effects. Oxymetazoline had a broad spectrum of functionality (antagonist, full and partial agonist) depending on the receptor examined. Compounds were serially diluted 4-fold to generate an 8 point dose response with each concentration performed in duplicate. Data shown for 5-HT_{2A} in panel B is from 5-HT_{2A} expressing cells treated with the indicated concentrations of oxymetazoline and then challenged with an EC_{80} concentration of reference agonist to reveal activity oxymetazoline antagonistic activity.

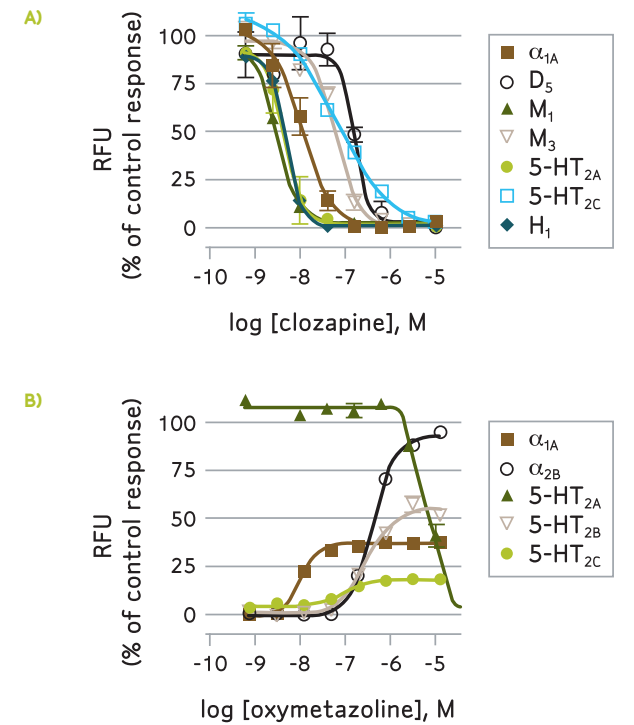
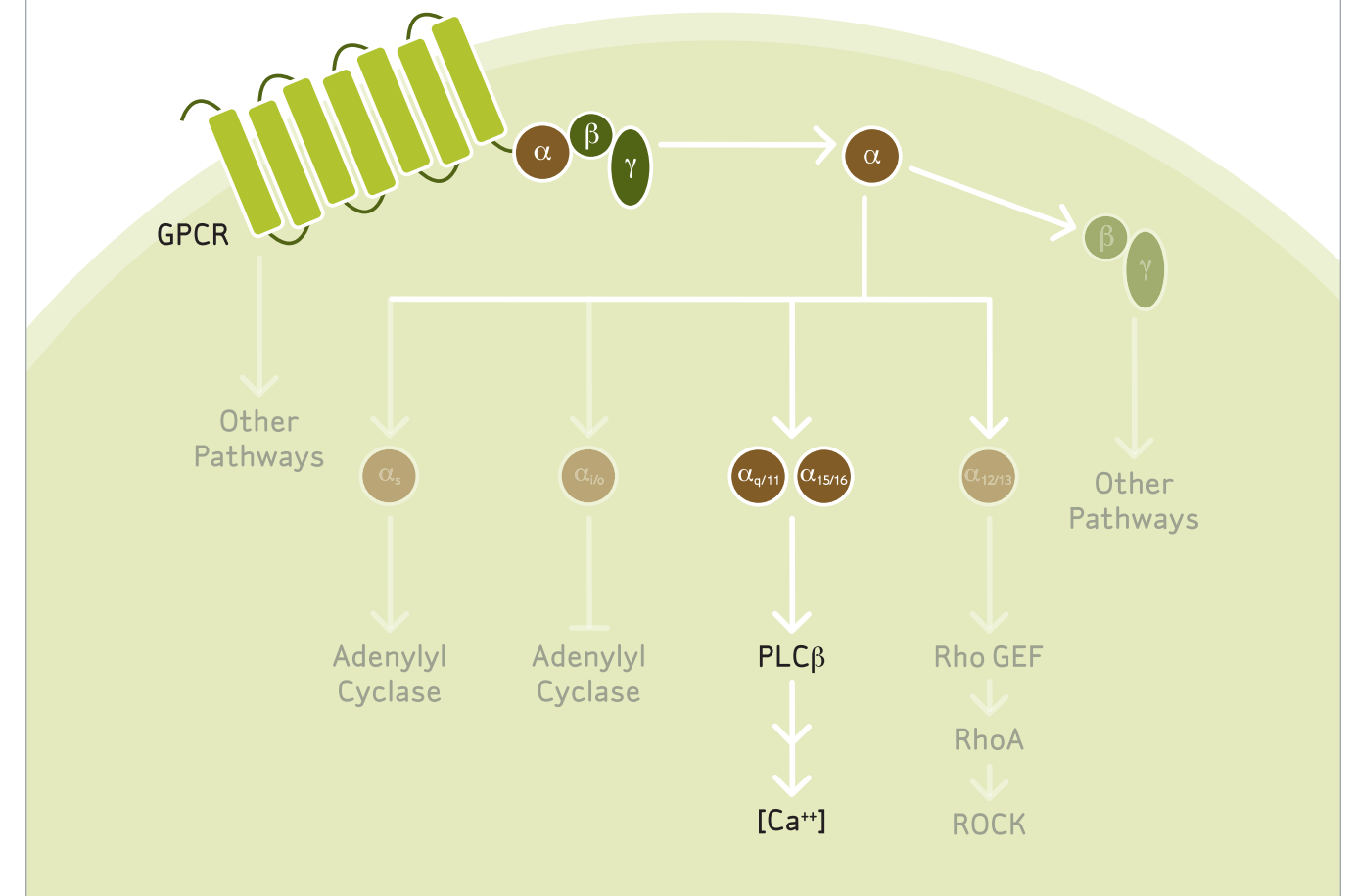


Figure 5. GPCRProfiler® Services Uses Millipore's Stable ChemiScreen™ Ca²⁺-Optimized GPCR Cell Lines to Provide a Standard Assay Setup and Common Readout for All Targets.



GPCRProfiler SERVICES ORDERING INFORMATION

Affordable Full Panel & Safety Panels

▲ = NEW
● = COMING SOON

Class	Ligand Type	GPCR Family	GPCR Target (Human unless noted)	
A	non-peptide	Acetylcholine (muscarinic)	M1	
A	non-peptide	Acetylcholine (muscarinic)	M2	
A	non-peptide	Acetylcholine (muscarinic)	M3	
A	non-peptide	Acetylcholine (muscarinic)	M4	
A	non-peptide	Acetylcholine (muscarinic)	M5	
A	non-peptide	Adenosine	A1	
A	non-peptide	Adenosine	A2B	
A	non-peptide	Adenosine	A3	
A	non-peptide	Adrenergic	α1A	
▲	A	non-peptide	Adrenergic	α1B
▲	A	non-peptide	Adrenergic	α1D
A	non-peptide	Adrenergic	α2A	
A	non-peptide	Adrenergic	α2B	
A	non-peptide	Adrenergic	β1	
A	non-peptide	Adrenergic	β2	
A	non-peptide	Adrenergic	β3	
A	peptide	Anaphylotoxin	C3aR	
A	peptide	Anaphylotoxin	C5aR	
A	peptide	Angiotensin	AT1	
▲	A	peptide	Apelin	APJ
A	peptide	Bombesin	BB1	
A	peptide	Bombesin	BB2	
A	peptide	Bombesin	BB3	
A	peptide	Bradykinin	B2	
B	peptide	Calcitonin	CGRP1	
C	non-peptide	Calcium sensor	CaS	
A	non-peptide	Cannabinoid	CB1	
A	non-peptide	Cannabinoid	CB2	
A	peptide	Chemoattractant	ChemR23 / CMLKR1	
A	peptide	Chemokine	CCR1	
A	peptide	Chemokine	CCR10	
A	peptide	Chemokine	CCR2B	
A	peptide	Chemokine	CCR3	
A	peptide	Chemokine	CCR4	
A	peptide	Chemokine	CCR5, rhesus macaque	
A	peptide	Chemokine	CCR6	
A	peptide	Chemokine	CCR7	
A	peptide	Chemokine	CCR8	
A	peptide	Chemokine	CCR9	
A	peptide	Chemokine	CX3CR1	
A	peptide	Chemokine	CXCR1 / IL-8a	
A	peptide	Chemokine	CXCR2 / IL-8b	
A	peptide	Chemokine	CXCR3	
A	peptide	Chemokine	CXCR4	
●	A	peptide	Chemokine	CXCR5
▲	A	peptide	Chemokine	CXCR6
A	peptide	Chemokine	XCR1 / GPR5	

Class	Ligand Type	GPCR Family	GPCR Target (Human unless noted)	
A	peptide	Cholecystokinin	CCK1 / CCKa	
A	peptide	Cholecystokinin	CCK2 / CCKb	
B	peptide	CRF Receptor	CRF1	
B	peptide	CRF Receptor	CRF2	
A	non-peptide	Dopamine	D1	
A	non-peptide	Dopamine	D2L	
A	non-peptide	Dopamine	D5	
A	peptide	Endothelin	ETA	
A	peptide	Endothelin	ETB	
A	non-peptide	Free Fatty Acid	FFA1 / GPR40	
A	non-peptide	Free Fatty Acid	FFA2 / GPR41	
A	non-peptide	Free Fatty Acid	FFA3 / GPR43	
C	non-peptide	GABA _B	GABAB1b	
A	peptide	Galanin	GAL1	
A	peptide	Galanin	GAL2	
▲	A	peptide	Ghrelin	Ghrelin / GHSR-1a / Growth Hormone Secretagogue Receptor
B	peptide	Glucagon	GIP	
B	peptide	Glucagon	GLP-1	
B	peptide	Glucagon	glucagon / GCG	
B	peptide	Glucagon	secretin receptor / SEC	
▲	C	non-peptide	Glutamate (metabotropic)	mGlu2
▲	A	peptide	Glycoprotein hormone	TSH / TSHR
A	peptide	GnRH	GnRH / LHRH	
A	non-peptide	Histamine	H1	
A	non-peptide	Histamine	H2	
A	non-peptide	Histamine	H3	
A	non-peptide	α-Ketoglutarate	OXGR1 / GPR99 / GPR80	
A	peptide	KISS1-derived peptide	KISS1 / GPR54	
A	non-peptide	Leukotriene	BLT1	
A	non-peptide	Leukotriene	CysLT1	
A	non-peptide	Leukotriene	CysLT2	
A	non-peptide	Lysophospholipid	LPA1 / EDG2	
A	non-peptide	Lysophospholipid	LPA3 / EDG7	
▲	A	non-peptide	Lysophospholipid	LPA5 / GPR92
A	non-peptide	Lysophospholipid	S1P1 / EDG1	

Class	Ligand Type	GPCR Family	GPCR Target (Human unless noted)	
A	non-peptide	Lysophospholipid	S1P2 / EDG5	
A	non-peptide	Lysophospholipid	S1P3 / EDG3	
A	non-peptide	Lysophospholipid	S1P4 / EDG6	
A	non-peptide	Lysophospholipid	S1P5 / EDG8	
▲	A	peptide	Mas-related gene	MRGPRD / MrgD
▲	A	peptide	Mas-related gene	MRGX1 / MRGPRX1
A	peptide	Mas-related gene	MRGX2 / MRGPRX2	
A	peptide	Melanin-concentrating hormone	MCHR1 / GPR24	
A	peptide	Melanin-concentrating hormone	MCHR2	
A	peptide	Melanocortin	MC2	
A	peptide	Melanocortin	MC4	
▲	A	peptide	Melanocortin	MC5
A	peptide	Motilin	Motilin Receptor / MTLR / GPR36	
A	peptide	Neuromedin U	NMU1	
▲	A	peptide	Neuromedin U	NMU2
A	peptide	Neuropeptide B / W	NPBW1 / GPR7	
A	peptide	Neuropeptide Y	Y2	
A	peptide	Neuropeptide Y	Y4	
A	peptide	Neurotensin	NTR1 / NTS1	
A	peptide	N-formylpeptide	FPR1	
A	peptide	N-formylpeptide	FPRL1 / AXL / HM63	
A	non-peptide	Nicotinic Acid	GPR109A	
A	peptide	Opioid	δ / OP1 / DOP / DOR	
A	peptide	Opioid	κ / OP2 / KPO / KOR	
A	peptide	Opioid	μ / OP3 / MOP / MOR	
A	peptide	Opioid	NOP / ORL1 / OP4	
A	peptide	Orexin	OX1	
A	peptide	Orexin	OX2	
A	peptide	Oxytocin	OT	
A	peptide	Peptide P518	GPR103 / QRFP	
▲	A	non-peptide	P2Y / Purinergic (metabotropic)	P2Y1
▲	A	non-peptide	P2Y / Purinergic (metabotropic)	P2Y2
▲	A	non-peptide	P2Y / Purinergic (metabotropic)	P2Y4
▲	A	non-peptide	P2Y / Purinergic (metabotropic)	P2Y11
A	non-peptide	Platelet Activating Factor	PAF	
A	peptide	Prokineticin	PK1 / PKR1	
A	peptide	Prokineticin	PK2 / PKR2	

Class	Ligand Type	GPCR Family	GPCR Target (Human unless noted)	
A	peptide	Prolactin-releasing peptide	PRP / PrRP / GPR10	
A	non-peptide	Prostanoid	DP	
A	non-peptide	Prostanoid	EP1	
A	non-peptide	Prostanoid	EP2	
A	non-peptide	Prostanoid	EP3	
A	non-peptide	Prostanoid	EP4	
A	non-peptide	Prostanoid	FP	
A	non-peptide	Prostanoid	IP1 / PF12	
A	non-peptide	Prostanoid	TP / TXA2 / PGH2	
A	peptide	Protease-activated	Trypsin-activated PARs	
A	peptide	Protease-activated	Thrombin-activated PARs	
B	peptide	PTH receptor	PTH1	
B	peptide	PTH receptor	PTH2	
A	non-peptide	Serotonin	5-HT1A	
A	non-peptide	Serotonin	5-HT2A	
A	non-peptide	Serotonin	5-HT2B	
A	non-peptide	Serotonin	5-HT2C	
●	A	non-peptide	Serotonin	5-HT6
▲	A	peptide	Somatostatin	sst2
A	peptide	Somatostatin	sst3	
A	peptide	Somatostatin	sst4	
A	peptide	Somatostatin	sst5	
A	non-peptide	SPC / LPC / proton-sensor	GPR68 / OGR1	
A	peptide	Tackykinin /neurokinin	NK1	
A	peptide	Tackykinin /neurokinin	NK2	
A	peptide	Tackykinin /neurokinin	NK3	
A	peptide	TRH	TRH	
A	peptide	Urotensin II	UT / UT1 / GPR14	
A	peptide	Vasopressin	V1A	
A	peptide	Vasopressin	V1B	
A	peptide	Vasopressin	V2	
B	peptide	VIP / PACAP	PAC1 long isoform / PACAP	
B	peptide	VIP / PACAP	VPAC1 / VIP1	
B	peptide	VIP / PACAP	VPAC2 / VIP2	

For the latest panel update or to request additional information or a quote, visit us at: www.millipore.com/gpcr or e-mail GPCRProfiler@millipore.com



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