Cerep

CELLULAR FUNCTIONAL GPCR ASSAYS

Functional GPCR assays are cell-based assays to measure agonist-like and antagonist activity of compounds. Various technologies are used: TR-FRET to determine cAMP concentration and IP1 levels, real time fluorescence to monitor calcium flux, cellular dielectric spectroscopy to measure impedance modulation. Large batches of cells, from which whole cells are frozen and stored for functional assays, are produced in-house. Cells are quality controlled before use. A novel patented host cell line was designed and constructed for Gi protein coupled receptors.

NON-PEPTIDE RECEPTORS

Family/assay	Ref.
ADENOSINE	
A_1	G127
A_{2A}	G002
A_{2B}	G003
A_3	G107
ADRENERGIC	
$alpha_{1A}$	G004
$alpha_{1B}$	G005
$alpha_{2A}$	G120
$alpha_{2B}$	G011
$alpha_{2C}$	G006
$beta_1$	G007
$beta_2$	G008
$beta_3$	G009
CALCIUM SENSING	
CaS	G010
CANNABINOID	
CB_1	G012
CB_2	G013
DOPAMINE	
D_1	G014
D_{2S}	G116
D_3	G016
$D_{4.4}$	G017
D_5	G018
FREE FATTY ACID	
FFA1 (GPR40)	G123
FFA2 (GPR43)	G132
FFA3 (GPR41)	G143
GABA	
$GABA_B$	G111
HISTAMINE	
H_1	G115
H_2	G020
H_3	G021
H_4	G125
LEUKOTRIENES	
BLT_1 (LTB_4)	G023
$CysLT_1$ (LTD_4)	G024
$CysLT_2$ (LTC_4)	G025

Family/assay	Ref.
LYSOPHOSPHOLIPID	
Lysophosphatidic acid	
LPA_2	G144
LPA_3	G145
Sphingosine 1-phosphate	
S_1P_2	G026
S_1P_3	G027
MELATONIN	
MT_1 (ML_{1A})	G136
MT_2 (ML_{1B})	G028
MUSCARINIC	
M_1	G029
M_2	G030
M_3	G031
M_4	G032
M_5	G033
PLATELET ACTIVATING FACTOR	
PAF	G034
PROSTANOID	
DP	G121
EP_1	G035
EP_2	G036
EP_3	G122
EP_4	G037
FP	G038
IP (PGI_2)	G039
TP (TXA_2/PGH_2)	G040
PURINERGIC	
$P2Y_1$	G041
$P2Y_2$	G108
$P2Y_4$	G042
$P2Y_6$	G043
SEROTONIN	
$5\text{-}HT_{1A}$	G044
$5\text{-}HT_{1B}$	G126
$5\text{-}HT_{1D}$	G112
$5\text{-}HT_{2A}$	G046
$5\text{-}HT_{2B}$	G047
$5\text{-}HT_{2C}$	G048
$5\text{-}HT_{4e}$	G049
$5\text{-}HT_6$	G050
$5\text{-}HT_7$	G051

PEPTIDE RECEPTORS

Family/assay	Ref.
ANGIOTENSIN II	
AT_1	G052
APELIN	
APJ (apelin)	G146
BOMBESIN	
BB_1	G147
BB_2	G053
BB_3	G054
BRADYKININ	
B_1	G055
B_2	G056
CALCITONIN	
CT (calcitonin)	G057
CALCITONIN GENE-RELATED PEPTIDE	
CGRP	G058
CHEMOKINES	
CCR1	G059
CCR2	G138
CCR3	G148
CXCR2 (IL-8B)	G113
CXCR4	G114
CHOLECYSTOKININ	
CCK_1 (CCK_A)	G062
CCK_2 (CCK_B)	G063
COMPLEMENT 5A	
C5a	G064
CORTICOTROPIN RELEASING FACTOR	
CRF_1	G065
$CRF_{2\alpha}$	G066
ENDOTHELIN	
ET_A	G067
ET_B	G068
N-*FORMYL* PEPTIDE	
fMLP	G069
GALANIN	
GAL_2	G070
GLUCAGON	
GLP-1	G072
GLP-2	G073
glucagon	G074
secretin	G075
GLYCOPROTEIN HORMONE	
TSH	G076
GROWTH HORMONE-RELEASING HORMONE	
GHRH	G071
MELANIN CONCENTRATING HORMONE	
MCH_1	G077
MCH_2	G078

Family/assay	Ref.
MELANOCORTIN	
MC_1	G079
MC_2	G080
MC_3	G081
MC_4	G082
MC_5	G083
MOTILIN	
motilin	G084
NEUROKININ	
NK_1	G085
NK_2	G086
NK_3	G087
NEUROPEPTIDE Y	
Y_1	G088
NEUROTENSIN	
NTS_1 (NT_1)	G089
OPIOID AND OPIOID-LIKE	
$delta_2$ (DOP)	G129
kappa (KOP)	G090
mu (MOP)	G091
NOP (ORL1)	G119
OREXIN	
OX_1	G092
OX_2	G093
PARATHYROID HORMONE	
PTH1	G130
PROKINETICIN	
PK_1	G117
PK_2	G118
PROTEINASE-ACTIVATED	
PAR1	G109
PAR2	G110
RELAXIN	
RXFP1	G094
SOMATOSTATIN	
sst_1	G095
sst_4	G096
sst_5	G097
THYROTROPIN RELEASING HORMONE	
TRH_1	G098
UROTENSIN-II	
UT	G099
VASOACTIVE INTESTINAL PEPTIDE	
PAC_1 (PACAP)	G100
$VPAC_1$ (VIP_1)	G101
$VPAC_2$ (VIP_2)	G102
VASOPRESSIN	
OT	G103
V_{1a}	G104
V_2	G105

cellular assay · new assay · new protocol · human

TESTING CONDITIONS

SUGGESTED TESTING

Primary screening on both agonist and antagonist effects at 1-10 µM in duplicate (2 wells), followed-up for IC_{50}/K_{Bapp} or EC_{50} determination (8 concentrations in duplicate (16 wells) when compound displays more than 50% inhibition or 50% of agonist response.

SAMPLE SIZE (including IC_{50} follow-up studies)

Assuming a molecular weight ≤ 500 g/mol and a testing concentration of 10 µM in duplicate (including a possible retest).

	SCREENING		SCREENING + FOLLOW UP [1]	
	WEIGHT (pre-weighed)	VOLUME (100% DMSO)	WEIGHT (pre-weighed)	VOLUME (100% DMSO)
INDIVIDUAL CATALOG ASSAYS				
1 to 3 assays	1 mg	25 µL@10 mM	1 mg	60 µL@10 mM
4 to 5 assays	1 mg	35 µL@10 mM	1 mg	70 µL@10 mM
6 to 10 assays	1 mg	50 µL@10 mM	1 mg	100 µL@10 mM
11 to 15 assays	1 mg	65 µL@10 mM	1 mg	110 µL@10 mM
16 to 20 assays	1 mg	75 µL@10 mM	1 mg	125 µL@10 mM
21 to 40 assays	1 mg	100 µL@10 mM	1.5 mg	250 µL@10 mM
41 to 50 assays	1 mg	150 µL@10 mM	2 mg	275 µL@10 mM
51 to 70 assays	1.5 mg	225 µL@10 mM	2.5 mg	400 µL@10 mM
71 to 100 assays	2 mg	300 µL@10 mM	3 mg	550 µL@10 mM
101 to 135 assays	2 mg	350 µL@10 mM	4 mg	650 µL@10 mM
136 to 150 assays	2.5 mg	400 µL@10 mM	4 mg	750 µL@10 mM
151 to 200 assays	3 mg	500 µL@10 mM	5 mg	1000 µL@10 mM
201 to 250 assays	3.5 mg	600 µL@10 mM	inquire	inquire

[1] *Assuming ~10% of test in EC_{50} or IC_{50}. Usually, for 1 curve: 30 µL@10 mM and + 25 µL@10 mM by additional EC_{50} or IC_{50} determination.*

REQUESTED COMPOUND INFORMATION

To reduce the registration time and ensure that all the appropriate information is available to start the study in a shortest possible timeframe, please use **Cerep compound submission form** [1] or MS Excel file [2], and provide the following **compound information**:

- **Name (compound ID) / Batch # / Molecular weight** [3] **/ Formula weight** [4] **/ Stock concentration / Stock solvent / Quantity / Unit** [5] **/ Form / Storage conditions / Solubility,** as well as **Plate ID / plate position** for compounds delivered in plates, **Comments** [6], and **Quotation number**.

  NOTE: Impurity and colored compounds might affect the results (compound color information is mentioned in the study report).

- **General remarks:**
  - **If compound(s) are supplied as a stock solution in plate(s)** (preferred format for any submission of 10 or more compounds), please leave columns 1 and 12 empty in a 96W plate. The 384W plate format is also acceptable with columns 1, 2, 23 and 24 empty. For any other plate format, please inquire.
  - **If compound(s) are not soluble in 100% DMSO**, please provide any useful information concerning the solubility of the compound. The following solvents are compatible with most of our assays: DMSO (Cerep standard), H_2O, Methanol, Tris/HCl 10 mM pH 7.4.
  - **Organic solvents such as acetone, chloroform, ether, acetonitrile, tetrahydrofuran and trifluoroacetic acid are not recommended** as they will significantly affect the results from many *in vitro* assays, even at very low concentrations.

WARNING: Cerep will apply the standard solubilization process when compounds are received at the testing site, unless special instructions are provided with the compounds.

Customized handling procedure of compounds can be accommodated, please inquire for pricing conditions.

PROTOCOL

A typical protocol for all cellular assays includes a minimum of 6-control wells (background, and maximal signal with and without vehicle) plus an 8-point dose-response of the relevant reference compound.

The reference compound for each assay is listed in each assay description. The historical average EC_{50} or IC_{50} value is also shown in each assay description.

▸ ANY OF OUR ASSAY PROTOCOLS CAN BE CUSTOMIZED: PLEASE INQUIRE

[1] *Cerep compound submission form will be emailed to you with your quotation. A copy can be requested from sales@cerep.com, or downloaded from Cerep website: www.cerep.com/Catalog Online*
[2] *Systematically required for studies of 10 compounds or more.*
[3] *Molecular weight (MW) of free acid or base form.*
[4] *Formula weight (FW) including salt form and/or hydrate form if applicable.*
[5] *mg?, mL?*
[6] *e.g. useful information such as sensitivity to light, stability or hygroscopicity issues.*

DELIVERABLES

Percent effect or percent inhibition (mean of replicates), individual values as percent of control, EC_{50} or IC_{50} value (calculated from a minimum 5 concentration testing), K_{Bapp}, Hill coefficient (nH), and plotted EC_{50} or IC_{50} curves.

DATA TURNAROUND

Complete data set is typically available within 3 weeks, after receipt of the compounds at the testing site (providing that we receive all available information to initiate the study).

Secure, password-protected data can be viewed on line as soon as they are produced, after scientific approval and QC-ed by an experienced technician.

▶ FOR MAJORITY OF ASSAYS, EXPRESSCREEN OPTION (5 BUSINESS DAYS) IS AVAILABLE: PLEASE INQUIRE

ASSAY CATALOG REFERENCES

In 2009, Cerep finalized one of the milestones in the industrialization process: the implementation of new referencing and supply chain management systems. The references of each of the assays have thus been simplified: **each reference will now be displayed as 4 digits.**

A correlation table between old and new assay references is available at **www.cerep.com** CATALOG ONLINE <http://www.cerep.com/Cerep/Users/pages/Catalog/Assay/catalog.asp>

QUESTIONS OR CONCERNS ?

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