Protocol of ADRB2 Cell-based Assay for High-throughput Screening

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TITLE: Protocol of CHO-ADRB2 Cell-based Assay for High-throughput Screening

ASSAY RFERENCES:

Assay Target	Cell Lines	Species	Tissue of Origin	Assay Readout	Assay Provider	Toxicity Pathway
ADRB2	CHO-K1	Hamster	Chinese hamster ovary	Homogeneous Time Resolved Fluorescence (HTRF)	Cisbio (ThermoFisher Scientific)	Gs-coupled

QUALITY CONTROL PRECAUTIONS:

1. Maintain cells below 85-90% confluence.
2. Feed or passage cells twice a week.

MATERIALS and INSTRUMENTS:

Supplies/Medium/Reagent	Manufacturer	Vender/Catalog Number
DMEM/F12	ATCC	ATCC (30-2006)
Fetal Bovine Serum, Qualified	GIBCO (ThermoFisher Scientific)	GIBCO (26140)
Penicillin/Streptomycin	GIBCO (ThermoFisher Scientific)	GIBCO (15140)
Puromycin	GIBCO (ThermoFisher Scientific)	GIBCO (A11138-03)
Recovery Cell culture Freezing Medium	GIBCO (ThermoFisher Scientific)	GIBCO (12648)
0.05% Trypsin-EDTA	GIBCO (ThermoFisher Scientific)	GIBCO (25300)
1536-well white solid plates	Greiner Bio-One	Greiner Bio-One (789173-F)
MULTIDROP COMBI	ThermoFisher Scientific	ThermoFisher Scientific
BioRAPTR FRD	Beckman Coulter	Beckman Coulter
Envision Plate Reader	Perkin Elmer	Perkin Elmer

CAMP - GS DYNAMIC KIT	Cisbio (Perkin Elmer)	Cisbio (62AM4PEJ (100,000 tests))
Isoproterenol hydrochloride (Agonist control compound)	Millipore Sigma	Millipore Sigma (I6504)
Propranolol hydrochloride (Antagonist control compound)	Millipore Sigma	Millipore Sigma (P0884)
3-Isobutyl-1-methylxanthine (IBMX)	Millipore Sigma	Millipore Sigma (I7018)
Ro-20-1724	Millipore Sigma	Millipore Sigma (557502)

PROCEDURE:

1. Cell handling:
1.1. Media Required:

Component	Growth Medium	Assay Medium	Thaw Medium	Freezing Medium
DMEM/F12	90%	90%	90%	-
Fetal Bovine Serum, Qualified	10%	10%	10%	-
Penicillin-Streptomycin (10,000 U/mL)	1%	1%	1%	-
Puromycin	10 µg/mL	-	-	-
Recovery Cell Culture Freezing Medium	-	-	-	100%

1.2. Thawing method:

1.2.1 Thaw a vial of cells in 9 mL of pre-warmed medium and seed them in T-175 flask at 2 x 10^6 cells.

1.3. Propagation method:

1.3.1 Trypsinize cells from the flask and centrifuge.
1.3.2 Add culture medium to the pellet and passage at 2-3 x 10^6 cells per T-225 flask.

2. Assay Protocol:

2.1. Harvest cells from the culture and re-suspend cells in assay medium at 0.25 x 10^6 cells/mL.

2.2. Dispense 1,000 cells/4 µL/well in 1536-well tissue treated white/solid bottom plates using Multidrop COMBI.

2.3. Incubate the assay plates for an overnight (18-20 hr) at 37°C and 5% CO_2.

2.4. Transfer 23 nL of the test compounds from the libraries and positive control to the assay plates through Pintool.

2.5. Add 1 µL of stimulation mixture (Prepare a mixture of 100 µM IBMX and 25 µM Ro-20-1724 in stimulation buffer provided in the Cisbio kit) and agonist stimulation mixture (Prepare a mixture of 100 µM IBMX, 25 µM Ro-20-1724, and 1.0 nM Isoproterenol hydrochloride in stimulation buffer provided in the Cisbio kit) to the specific wells of the assay plates as shown in the plate map using BioRAPTR FRD.

2.6. Centrifuge the assay plates at 1000 rpm for 15 sec.

2.7. Incubate the assay plates for 0.5 hr at room temperature.

2.8. Add 2.5 µL of cAMP-d2 working solution to all the wells of the assay plates using BioRAPTR FRD. (Prepare 20X stock solution of cAMP-d2 by reconstituting cAMP-d2 with 5 mL distilled water and then dilute one volume of 20X stock solution in 19 volumes of Lysis & Detection buffer.)

2.9. Add 2.5 µL of Anti-cAMP-Cryptate to all the wells of the assay plates using BioRAPTR FRD. (Prepare 20X stock solution of Anti-cAMP-Cryptate reconstituting Anti-cAMP-Cryptate with 5 mL distilled water and then dilute one volume of 20X stock solution in 19 volumes of Lysis & Detection buffer.)

2.10. Incubate the assay plates at room temperature for 1 hr.

2.11. Read the fluorescence intensity using Envision plate reader with an excitation wavelength at 340 nm and emission at two different wavelengths; 665nm and 615nm. Final data are expressed as ratio of 665nm/620 nm.

3. Assay Performance:

ADRB2 (Antagonist mode)	**Online Screening (Mean ± SD)**
AC_{50}	5.60 ± 2.4 nM (n = 406)
S/B	2.02 ± 0.21
CV (%)*	7.91 ± 2.93 (n = 408)
Z’	0.38 ± 0.24

*CV values shown represent average of DMSO-only column from all the assay plates.