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READY-TO-ASSAY™ CALCIUM-OPTIMIZED CELLS
HUMAN RECOMBINANT α_{2B} ADRENOCEPTOR

CATALOG NUMBER:	HTS157F	**QUANTITY:**	1 vial, 1 mL
LOT NUMBER:		**CONCENTRATION:**	1 x 10^7 viable cells/mL

BACKGROUND:

Millipore's Ready-To-Assay™ Calcium-Optimized Cells are GPCR-expressing cell lines that are designed for simple, rapid calcium assays with no requirement for culturing cells. The user simply thaws the cells with maximal viability, dispenses into assay plates, and assays for calcium response the next day.

The Ready-To-Assay™ cells are derived from ChemiScreen™ calcium-optimized stable cell lines, which express the GPCR target of interest at high levels on the cell surface, in a host cell line containing high levels of the promiscuous Gα15 protein to couple the receptor to the calcium signaling pathway. The Ready-To-Assay™ cells are prepared by chemical treatment at a concentration optimized for effective growth arrest while maintaining high viability (>80%) after thawing and overnight plating. Pharmacological functionality of the Ready-To-Assay™ cells is identical to that of the originating GPCR cell line.

The endogenous catecholamines epinephrine and norepinephrine have profound effects on smooth muscle activity, cardiac function, carbohydrate and fat metabolism, hormone secretion, neurotransmitter release, and central nervous system actions. These activities are mediated by GPCRs belonging to two subfamilies, the α- and β-adrenoceptors (Bylund *et al.*, 1994). The α_2 adrenergic receptor subfamily members, consisting of α_{2A}, α_{2B}, and α_{2C}, couple primarily to G_i to inhibit cAMP production, and play an important role in regulation of cardiovascular and CNS function. Experiments with α_{2B}-selective agonists and mice lacking α_{2B} demonstrate that α_{2B} plays a role in salt-induced hypotension. Also, the difficulty in breeding homozygous α_{2B}-KO mice indicates the gene may also play an as-yet-unknown role in development or reproduction. (Kable *et al.*, 2000). Millipore's cloned human α_{2B} -expressing cell line is made in the Chem-1 host cells, an adherent cell line that supports high levels of recombinant α_{2B} expression on the cell surface and contains high levels of promiscuous G protein to couple the receptor to the calcium signaling pathway. The untreated human α_{2B} -Chem-1 cell line and the Ready-To-Assay™ human α_{2B} cells have equivalent EC50s for oxymetazoline.

APPLICATIONS:

Calcium flux assay

SPECIFICATIONS:

	EC50 for Oxymetazoline (μM)	Maximum Signal (RFU)	Z'
Ready-To-Assay Cells	1.05	5004	0.61
Continuous Passage Cells	0.87	5877	0.80

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HOST CELLS: Chem-1 an adherent cell line expressing a recombinant promiscuous G-protein.

TRANSFECTION: Full-length human ADRA2B transcript cDNA encoding α_{2B} (Accession Number: NM_000682)

PLATING MEDIA:
DMEM with 4.5 g/L glucose and 4 mM glutamine (Millipore SLM-020-A)
10% heat-inactivated FBS
1x Nonessential amino acids (from 100x stock, Millipore TMS-001-C)
10mM HEPES (from 1 M HEPES, Millipore TMS-003-C)
100 U/mL Pen-Strep (from 100x stock, Millipore TMS-AB2-C)

PRESENTATION:

Cells are frozen at 1 x 10^7 cells/mL in DMEM/20% fetal bovine serum/100 U/ml penicillin and streptomycin/10% DMSO.

STORAGE:

Place cells in liquid nitrogen immediately upon receipt. Maintain frozen in liquid nitrogen for up to 5 years.

ASSAY PROTOCOL:

1) Thaw cells rapidly by removing from liquid nitrogen and immediately immersing in a 37ºC water bath. Immediately after ice has thawed, sterilize the exterior of the vial with 70% ethanol.
2) Transfer contents of the vial to a sterile 15 mL conical tube. Add 10 mL prewarmed plating media to the cells and mix gently to resuspend cells. Centrifuge at 200 x g. Remove all but 0.5 mL media.
3) Resuspend cells to 0.5 x 10^6 cells/mL in plating media. Dispense the cell suspension into a 96-well assay plate at 200 µL per well to obtain a density of approximately 1 x 10^5 cells/well.
4) Place the assay plate in a humidified 37°C incubator with 5% CO_2.
5) The cells may be assayed 16-24 hours after plating. It is recommended to wash the cells with assay buffer at least once prior to addition of loading dye.

REFERENCES:

Bylund DB *et al.* (1994). IV. International Union of Pharmacology nomenclature of adrenoceptors. *Pharmacol. Rev.* 46: 121-136.

Kable JW *et al.* (2000) In vivo gene modification elucidates subtype-specific functions of α_2-adrenergic receptors. *J. Pharmacol. Exp. Ther.* 293: 1-7.

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READY-TO-ASSAY™ CALCIUM-OPTIMIZED CELLS
HUMAN RECOMBINANT α_{2B} ADRENOCEPTOR

Product No. HTS157F

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