CHO-K1/EDNRA Cells Ready-to-Use™

Technical Manual No. TM0367 **Version 02062009**

I. PRODUCT INFORMATION

Catalog No: M00157 Lot.No: D201901

Host Cell: CHO-K1 Expressed Gene: Genbank Accession Number NM_001957

Quantity: 1×10^7/vial Mycoplasma Status: Negative

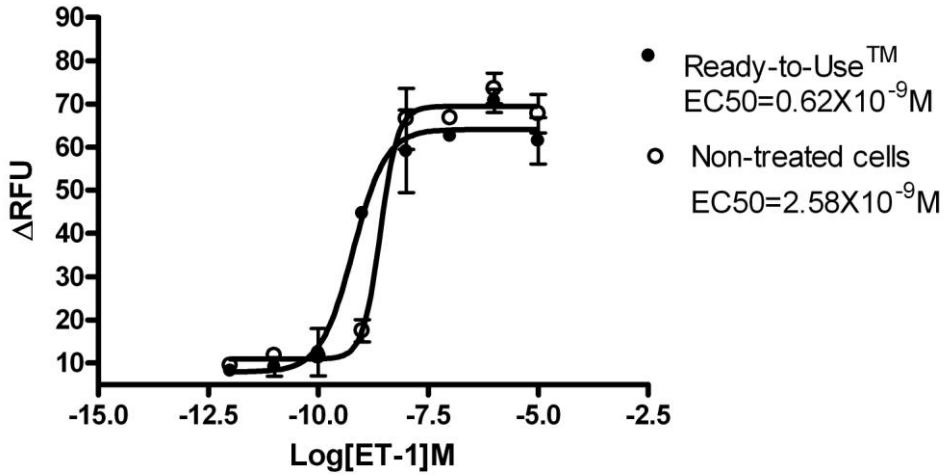
Storage: Liquid nitrogen Freeze Medium: Culture medium plus 10% DMSO

Culture Medium: F12, 10% FBS, 100 IU/ml penicillin, 100 µg/ml streptomycin, 250 µg/ml Zeocin

II. BACKGROUND

In mammals, the endothelin (ET) family comprises three endogenous isoforms: ET-1, ET-2, and ET-3. These endothelium-derived peptides perform their functions via two endothelin receptors, classified as EDNRA and EDNRB. EDNRA receptors are mainly localized in the vascular smooth muscle cells, where they are the predominant sub-type, and mediate vasoconstriction and proliferation. Selective EDNRA antagonists are effective in the treatment of heart failure, essential hypertension, pulmonary hypertension, and atherosclerosis.

III. REPRESENTATIVE DATA



This diagram depicts the calcium dose-response of ET-1 stimulation of CHO-K1/EDNRA Cells Ready-to-Use™ (●) and non-treated cells (○). The resulting increase in intracellular calcium was measured on a FlexStation fluorescent plate reader. Data points represent the average +/- standard deviation of triplicate determinations.

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IV. ASSAY PROCEDURE

Calcium flux assay of CHO-K1/EDNRA receptor cell with FLIPRP® Calcium 4 Assay Kit
1. Prepare ET-1 addition plates in advance of assay.
2. Seed cells at a density of 2.0×10^4 cells/well in a 384-well black plate.
3. Incubate the cells overnight at 37°C 5% CO_2.
4. Remove cell plates from the incubator and add an equal volume of Calcium4 Loading Buffer to each well (25 µl per well for 384-well plate).
5. Incubate the plate in the dark for one hour at 37°C in and then for 30 minutes at room temperature.
6. Add 5X working concentration of ET-1 in HEPES buffered HBSS solution (PH=7.4) to cell plate for 12.5 µl/well (assumes a 384-well plate).
7. Read with FlexStationIII, FLIPR or a similar fluorescence plate reader. The signal should be measured for two minutes. Add ET-1 at the 20th second of the measurement.

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