

Caution: For Laboratory Use. A research reagent for research purposes only

Human Melanocortin MC4 Receptor, γ -Irradiated Frozen Cells

Product No.: ES-191-CF

Lot No.: 1001

Material Provided

Cells: 1 x 1 mL frozen aliquot

Format: ~2.5 x 10⁶ cells / mL in Ham's F12, 10% FBS with 10 % DMSO

Product Information

Cellular Background: CHO-K1

Frozen cells info: Frozen recombinant, γ -irradiated CHO-K1 cells expressing the Human Melanocortin MC4 receptor.

DNA Sequence: Identical to coding sequence of GenBank NM_005912.2.

Corresponding Protein Sequence: Identical to GenBank NP_005903.2.

Storage Conditions: Store in liquid nitrogen (vapor phase) immediately upon receipt, or maximum 15 days at -80°C. cAMPZen[®] is designed for single use only. Do not refreeze.

Quality Control

EC₅₀ for a reference agonist is determined using a LANCE[®] cAMP assay (Figure 1). Mycoplasma test is performed using MycoAlert[®] Mycoplasma detection kit. We certify that these results meet our quality release criteria.

NDP- α -MSH - (EC₅₀): 0.16 nM

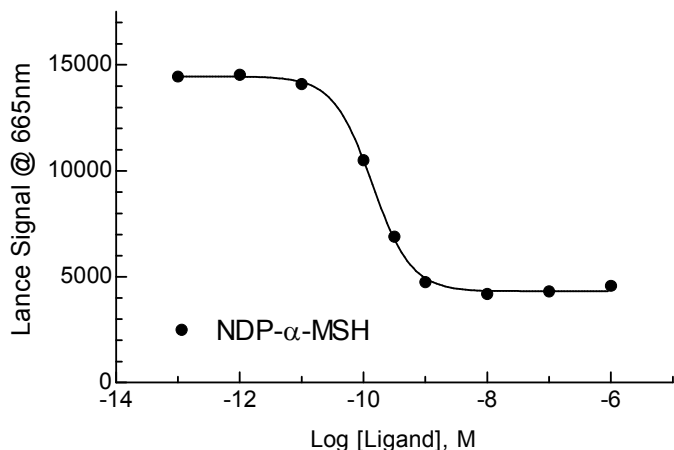
Mycoplasma: This cell line tested negative for Mycoplasma.

Recommended Use and Thawing Conditions

Assay Medium: HBSS 1X (without phenol red); 5 mM HEPES; BSA-stabilizer (7.5% solution); 0.5 mM IBMX (pH 7.4).

Thawing Cells: Using appropriate personal protective equipment, place the frozen aliquot in a 37°C water bath and agitate until its content is thawed completely. Immediately remove from water bath, transfer content to 10 mL assay medium and centrifuge (150 x g, 5 min). Resuspend cell pellet in appropriate volume of assay medium for use in the selected LANCE[®] cAMP assay (see LANCE[®] cAMP Assays section).

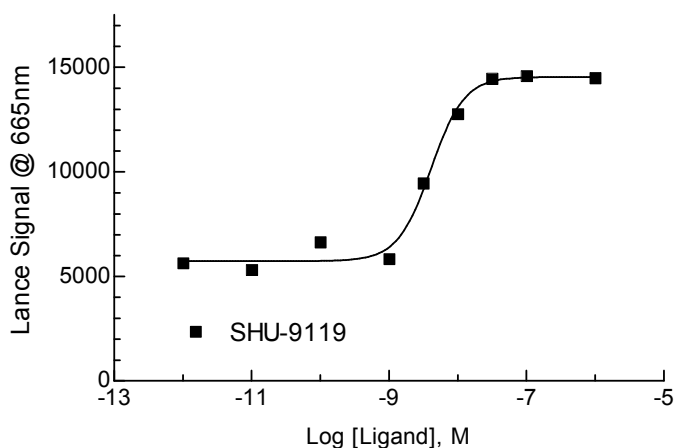
Typical Product Data



Agonist	EC ₅₀ (M)
NDP-α-MSH	1.4 x 10 ⁻¹⁰

Figure 1: Agonist Response in LANCE[®] cAMP assay

An agonist dose-response experiment was performed in 384-well format using 2 500 cells/well. Cell stimulation was performed for 30 min at room temperature. Reader: EnVision[®]. Data from a representative experiment are shown.



Antagonist	IC ₅₀ (M)
SHU-9119	4.1 x 10 ⁻⁹

Figure 2: Antagonist Response in LANCE[®] cAMP assay

An antagonist dose-response experiment was performed in 384-well format using 2 500 cells/well and 0.5 nM NDP-α-MSH (reference agonist). Cell stimulation was performed for 10 min at room temperature. Reader: EnVision[®]. Data from a representative experiment are shown.

Table 1. References of compounds used for functional characterization

Name	Provider	Cat n°	Working Stock Solution
(Nle4, D-Phe7)- α -MSH (NDP- α -MSH)	Bachem	H-1100	10 mM in water
SHU-9119	Bachem	H-3952	10 mM in water

LANCE[®] cAMP Assays

LANCE[®] Ultra cAMP - please refer to the user manual included with the LANCE[®] Ultra cAMP kits:

TRF0262 – 1 000 Assay points

TRF0263 – 10 000 Assay points

TRF0264 – 50 000 Assay points

LANCE[®] cAMP - please refer to the user manual included with the LANCE[®] cAMP kits:

AD0262 – 500 Assay points

AD0262E – 1 000 Assay points

AD0263 – 10 000 Assay points

AD0264 – 50 000 Assay points

Suggested Materials and Instrumentation

Please visit our website

www.perkinelmer.com/CellLines

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