Human Recombinant OX40 Stable Cell Line

Cat. No. M00608 **Version 06152017**

I. INTRODUCTION

Catalog Number: M00608
Cell Line Name: GS-H2/OX40
Gene Synonyms: CD134, ACT35, TNFRSF4, IMD16
Expressed Gene: NM_003327.3; no expressed tags
Host Cell: GS-H2
Quantity: Two vials of frozen cells (1×10^6 per vial)
Stability: 20 passages
Application: *in vitro* functional assay
Freeze Medium: 95% complete growth medium, 5% DMSO
Complete Growth Medium: MEM, 10% FBS
Culture Medium: MEM, 10% FBS, 2 μg/ml Puromycin, 200 μg/ml Hygromycin B
Mycoplasma Status: Negative
Functional Performance: For OX40L protein, Signal / Background (S/B) > 3, average EC_{50} of OX40L historic data in GenScript is 100.12 ng/ml.
Storage: Liquid nitrogen immediately upon receipt

II. BACKGROUND

OX40 (CD134) and its binding partner, OX40L (CD252), are members of the TNFR/TNF superfamily and are expressed on activated $CD4^+$ and $CD8^+$ T cells as well as a number of other lymphoid and non-lymphoid cells. The binding of CD252 (OX40L) on TH cells to OX40 activates antigen presenting cells and induces a variety of downstream effects.

III. REPRESENTATIVE DATA

- **Protein Expression Validation**

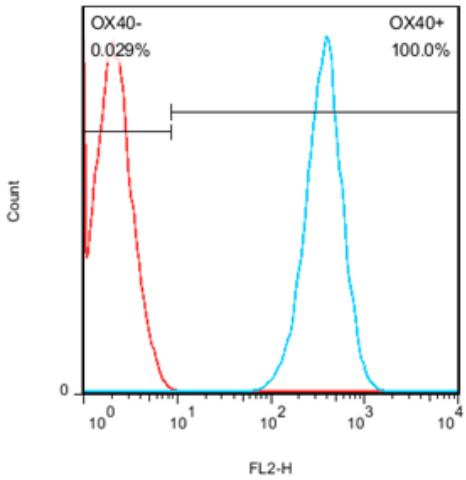

Figure 1. Flow cytometry analysis of OX40 expression in GS-H2/OX40 cells. Red: GS-H2, Blue: GS-H2/OX40.

- **Validation by *in vitro* Functional Assay**

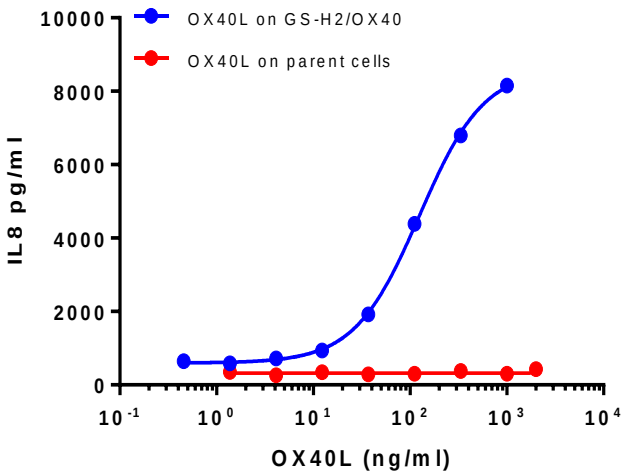

Figure 2. Functional analysis of OX40L protein on GS-H2/OX40 cells

- **Stability Validation**

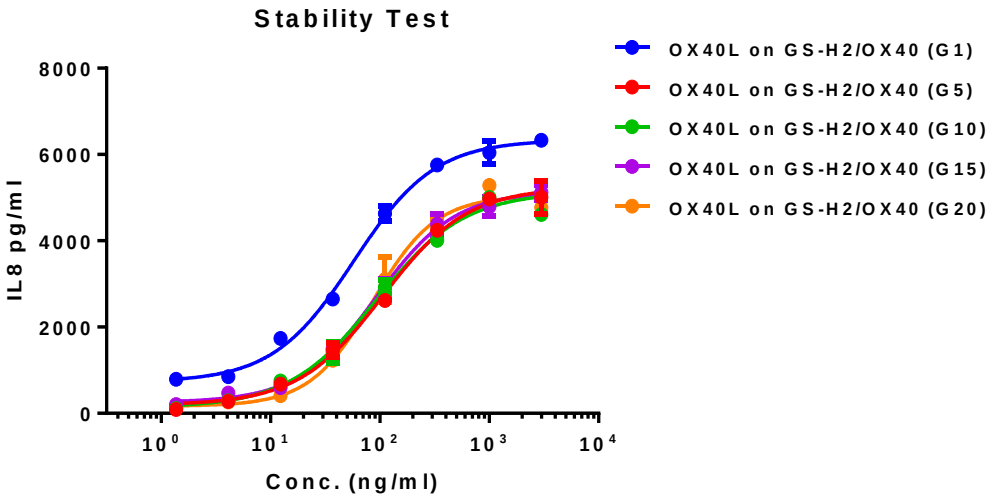

Figure 3. Stability testing of GS-H2/OX40 cells by *in vitro* functional assay

IV. THAWING AND SUBCULTURING

Thawing Protocol

1. Remove the vial from liquid nitrogen tank and thaw cells quickly in a 37°C water-bath.
2. Just before the cells are completely thawed, decontaminate the outside of the vial with 70% ethanol and transfer the cells to a 15 ml centrifuge tube containing 9 ml of complete growth medium.
3. Pellet cells by centrifugation at 200 x g for 5 min, and remove the medium.
4. Resuspend the cells in complete growth medium.
5. Transfer the cell suspension to a 10 cm dish with 10 ml of complete growth medium.
6. Grow the cells in incubator with 37°C, 5 % CO_2.
7. Add antibiotic in the following day.

Sub-culturing Protocol

1. Remove the culture medium from cells.
2. Wash cells with PBS (pH=7.4) to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 ml of 0.25% (w/v) Trypsin-EDTA (Gibco, Cat No. 25200) solution into 10 cm dish and observe the cells under an inverted microscope until cell layer is dispersed (usually within 3 to 5 minutes).
   Note: To avoid cells clumping, do not agitate the cells by hitting or shaking the dish while waiting for the cells detach. If cells are difficult to detach, please place the dish in 37°C incubator for ~2 minutes.
4. Add 6.0 to 8.0 ml of complete growth medium into dish and aspirate cells by gently pipetting.
5. Centrifuge the cells at 200 x g for 5minutes, and remove the medium.
6. Resuspend the cells in culture medium and add the cells suspension to new culture dish.
7. Grow the cells in incubator with 37°C, 5 % CO_2.

Subcultivation Ratio: A subcultivation ratio of 1:4 to 1:8 is recommended

Medium Renewal: Every 2 to 3 days

V. REFERENCES

1. Michael Croft, Takanori So, Wei Duan, and Pejman Soroosh. The Significance of OX40 and OX40L to T cell Biology and Immune Disease. Immunol Rev. 2009 May: 229(1): 173-191.
2. Pratima Bansal-Pakala, Beth S. Halteman, Mary Huey-Yu Cheng and Michael Croft. Costimulation of CD8 T Cell Responses by OX40. J Immunol. 2004 April:172(8): 4821-4825.
3. Stefanie N. Linch, Michael J. McNamara, and William L. Redmond. OX40 Agonists and Combination Immunotherapy: Putting the Pedal to the Metal. Front Oncol. 2015; 5: 34.

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