

# Human Recombinant CD40 Stable Cell Line Cat. No. M00606

### Version 04282015

#### I. INTRODUCTION

Catalog Number: M00606 Cell Line Name: GS-H2/CD40 Gene Synonyms: Bp50, CDW40, TNFRSF5, p50 Expressed Gene: Codon Optimized from NM\_001250.4; no expressed tags Host Cell: GS-H2 Quantity: Two vials of frozen cells (1×10<sup>6</sup> 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 Mycoplasma160: Negative Functional Performance: For CD40L protein, Signal / Background (S/B) > 3 Storage: Liquid nitrogen immediately upon receipt

#### II. BACKGROUND

Cluster of differentiation 40, CD40 is a costimulatory protein found on antigen presenting cells and is required for their activation. The binding of CD154 (CD40L) on TH cells to CD40 activates antigen presenting cells and induces a variety of downstream effects.

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**Protein Expression Validation** 

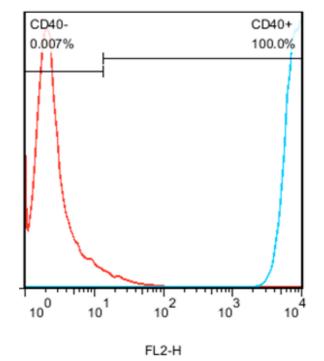


Figure 1. Flow cytometry analysis of CD40 expression in GS-H2/CD40 cells. Red: GS-H2, Blue: GS-H2/CD40.

Validation by in vitro Functional Assay

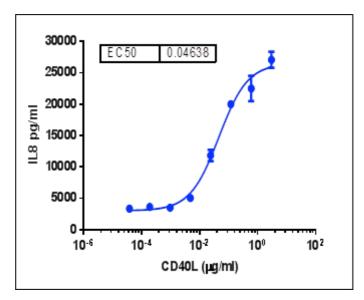


Figure 2. Functional analysis of CD40L protein on GS-H2/CD40 cells

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#### **III. 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  $37^{\circ}C$ , 5 % CO<sub>2</sub>.
- 7. Add antibiotic in the following day.

#### Sub-culturing Protocol

- 1. Centrifuge the cells at 200 x g for 5min, and remove the medium.
- 2. Resuspend the cells in culture medium and add the cells suspension to new culture dish.
- 3. Grow the cells in incubator with 37°C, 5 % CO<sub>2</sub>.

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

Medium Renewal: Every 2 to 3 days

#### **IV. REFERENCES**

1. Chatzigeorgiou A, Lyberi M, Chatzilymperis G, Nezos A, Kamper E. CD40/CD40L signaling and its implication in health and disease. BioFactors (Oxford, England). 2009, 35(6): 474–83.

2. Raul Elgueta, Micah J. Benson, Victor C. de Vries, Anna Wasiuk, Yanxia Guo, and Randolph J. Noelle. Molecular mechanism and function of CD40/CD40L engagement in the immune system. Immunol Rev. 2009 May: 229(1).

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