Data Sheet
LIGHT CHO Recombinant Cell Line
Catalog # AMS.79262

Background
LIGHT (TNFSF14) is a key component of the communication system that controls the responses of T-Cells. LIGHT activates two cell surface receptors, the Herpesvirus Entry Mediator (HVEM) and the Lymphotoxin-beta Receptor (LTβR). The LIGHT-HVEM axis is an important costimulatory pathway for T cells, it has been shown to activate NF-κB; LIGHT/HVEM pair are co-stimulatory immune checkpoint molecules for cancer immunotherapy, LIGHT expressing cells have been shown to be more potent than the soluble LIGHT for activating HVEM expressed on T cell surface.

Description
Recombinant stable clonal CHO cell line constitutively expressing full length human LIGHT protein, also known as TNFSF14, (Genbank #NM_003807). Surface expression is confirmed by flow cytometry.

Application
This cell line is useful for LIGHT binding assays, flow cytometry, or for screening for LIGHT antibodies. Also useful for immune checkpoint co-stimulatory assay when co-cultured with HVEM/NF-κB-luciferase/Jurkat reporter cell line.

Host Cell
CHO K1 cell line, Chinese Hamster Ovary

Format
Each vial contains ~ 2 x 10^6 cells in 1 ml of 10% DMSO in FBS.

Storage
Store in liquid nitrogen immediately upon receipt.

Cell Culture

Thaw Medium 3 (AMSBIO, #60186): Ham's F-12 medium (Hyclone # SH30526.01)supplemented with 10% FBS (Life technologies #26140-079), 1% Penicillin/Streptomycin
(Hyclone SV30010.01).

Growth Medium 3D (AMSBIO, #AMS.79539): Thaw Medium 3 (AMSBIO, #60186) plus 1mg/ml G418 (Thermo Fisher, #11811031).

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AMSBIO | www.amsbio.com | info@amsbio.com

UK & Rest of the World
184 Park Drive, Milton Park
Abingdon OX14 4SE, UK
T: +44 (0)1235 828 200
F: +44 (0) 1235 820 482

North America
1035 Cambridge Street,
Cambridge, MA 02141
T: +1 (617) 945-5033 or
T: +1 (800) 987-0985
F: +1 (617) 945-8218

Germany
Bockenheimer Landstr. 17/19
60325 Frankfurt/Main
T: +49 (0) 69 779099
F: +49 (0) 69 13376880

Switzerland
Centro Nord-Sud 2E
CH-6934 Bioggio-Lugano
T: +41(0) 91 604 55 22
F: +41(0) 91 605 17 85

Recommended Culture Condition

Frozen Cells: Prepare a 50 ml conical tube with 10 ml of pre-warmed Thaw Medium 3 (**no G418**). Quickly thaw cells in a 37°C water bath with constant and slow agitation. Clean the outside of the vial with 70% ethanol and immediately transfer the entire content to Thaw Medium 3 (**no G418**). Avoid pipetting up and down, and gently rock the conical tube.

Spin the cells down at 150 x g for 5 minutes. Discard the medium and re-suspend the cell pellet in fresh Thaw Medium 3 (**no G418**). Transfer the entire content to a T25 flask to distribute the cells. Incubate the cells in a humidified 37°C incubator with 5% CO_2. After 48-72 hours of incubation, change to fresh Thaw Medium 3 (**no G418**), without disturbing the attached cells. Continue to change the medium every 2-3 days until the cells reach desired confluency. If slow cell growth occurs during resuscitation, increase FBS to 15% for the first week of culture. Switch to Growth Medium 3D (**containing G418**) after the first passage.

Subculture: When cells reach 90% confluency, remove the medium and GENTLY wash once with PBS (without Magnesium or Calcium). These cells are loosely adherent and detach easily so do not re-suspend the PBS directly onto the cell surface. Treat cells with 2 ml of 0.25% trypsin/EDTA and incubate for 2-3 minutes at 37°C. After confirming cell detachment by light microscopy, add 10 ml pre-warmed Growth Medium 3D and gently pipette up and down to dissociate cell clumps. Transfer cells to a 15 ml conical tube and centrifuge at 200 x g for 5 minutes. Remove the medium and re-suspend cells in 10 ml of pre-warmed Growth Medium 3D. Dispense 5 ml of the cell suspension into a new T75 flask containing 20 ml pre-warmed Growth Medium 3D. Incubate cells in a humidified 37°C incubator with 5% CO_2. Freeze cells in freezing medium (10% DMSO in FBS) when cells reach 90% confluency. Cells have been demonstrated to be stable for at least 15 passages; BPS recommends preparing frozen stocks so cells are not used beyond passage 20.

Mycoplasma Testing

This cell line has been screened using the MycoAlert™ Mycoplasma Detection Kit (Lonza, #LT07-118) to confirm the absence of Mycoplasma contamination. MycoAlert Assay Control Set (Lonza, #LT07-518) was used as a positive control.

Application References

1. Chen, *et.al.* (2013) Molecular mechanisms of T cell co-stimulation and co-inhibition. *Nat Rev Immunol.* 2013 April ; **13(4):** 227–242.
2. Steinberg, *et.al.* (2014) The Signaling Networks of the Herpesvirus Entry Mediator (TNFRSF14) in Immune Regulation. *Immunol Rev.* 2011 November; **244(1):** 169–187.
3. Rio, *et.al.* (2014) Therapeutic blockade of LIGHT interaction with HVEM and LTβR attenuates in vivo cytotoxic allogeneic responses. *Transplantation.* 2014 December 15; **98(11):** 1165–1174.

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F: +1 (617) 945-8218

Germany
Bockenheimer Landstr. 17/19
60325 Frankfurt/Main
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F: +49 (0) 69 13376880

Switzerland
Centro Nord-Sud 2E
CH-6934 Bioggio-Lugano
T: +41(0) 91 604 55 22
F: +41(0) 91 605 17 85

Quality Assurance

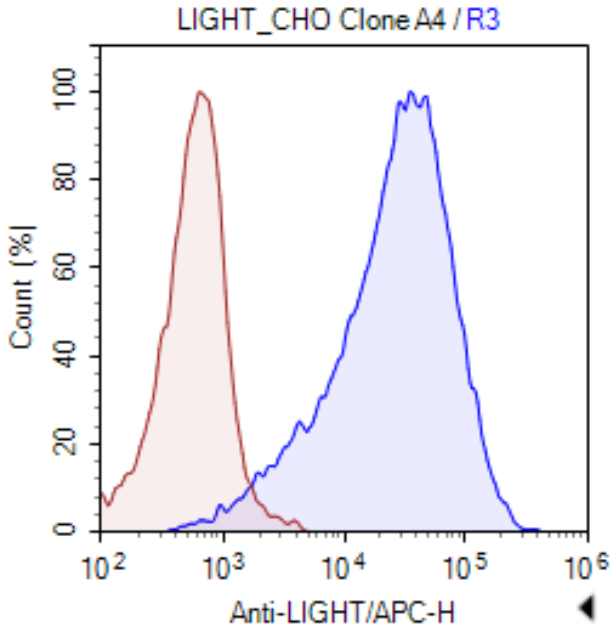

Figure 1. Expression of LIGHT validated by flow cytometry. Flow cytometry showed APC-conjugated anti-human LIGHT antibody (Abcam, #ab155363) detects LIGHT-positive clonal population (clone A4) (blue), using wild-type CHO cells as a negative control (red).

Vector and Sequence

Human LIGHT (NM_003807) was cloned into pIRESneo.

MEESVVRPSVFVVDGQTDIPFTRLGRSHRRQSCSVARVGLGLLLLLMGAGLAVQGWFLLQLH
WRLGEMVTRLPDGPAGSWEQLIQERRSHEVNPAAHLTGANSSLTGSGGPLLWETQLGLAFLR
GLSYHDGALVVTKAGYYYIYSKVQLGGVGCPLGLASTITHGLYKRTPRYPEELELLVSQQSPCG
RATSSSRVWWDSSFLGGVVHLEAGEKVVVRVLDERLVRLRDGTRSYFGAFMV

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F: +49 (0) 69 13376880

Switzerland
Centro Nord-Sud 2E
CH-6934 Bioggio-Lugano
T: +41(0) 91 604 55 22
F: +41(0) 91 605 17 85

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