# Certificate of Analysis

**Product Cat. No.:** M00567

**Host Cell:** CHO-K1

**Target gene:** Mouse PD-L1

**Shipping Conditions:** Dry ice

**Lot Number:** B30251703


860 Centennial Ave., Piscataway, NJ 08854, USA
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## Stable Cell Line Information

**Recommended Cell Culture Medium:** F-12K (Gibco, Cat. #21127-022), FBS (Gibco, Cat. #10099-141), and 300μg/ml GENETICIN* (Gibco, Cat. #10131-027)

**Freeze Medium:** 95% complete growth medium, 5% (V/V) DMSO

**Description:** One stable subline using CHO-K1 as the host will be established to overexpress Mouse PD-L1.

**QC:** FACS

**Mycoplasma Test:** Negative**

***** *Concentration used for selection was 600 μg/ml GENETICIN.*

****** *Our PCR mycoplasma test covers 160 of the most common species of mycoplasma, with sufficient sensitivity and specificity.*

**Notice to Purchaser:**

GenScript stable cell line products are to be used for research purposes only, not intended for use in humans. GenScript products may not be transferred to third parties or used to manufacture commercial products without written approval. Use of this product is also subject to compliance with the licensing requirements.

Cells will be stored at GenScript for additional 6 months after cells been shipped. If client fails to recover the cells, GenScript provides a replacement for once with shipping charge only.


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## QC Data

### 1. Validation of Flow Cytometry

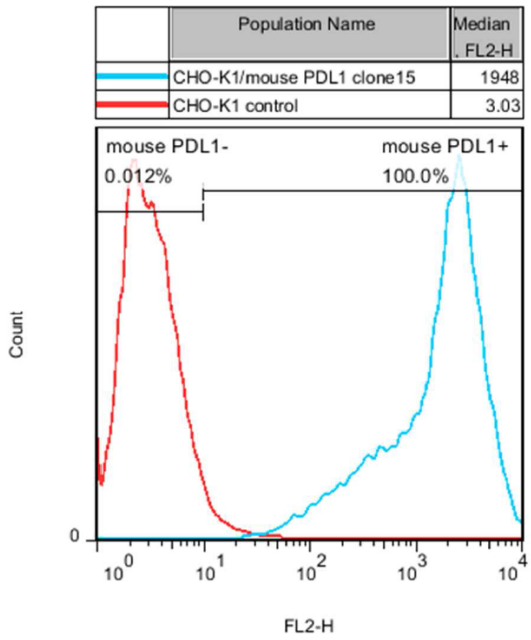


**Figure 1.** FACS analysis of Mouse PD-L1 in CHO-K1/Mouse PD-L1 clone15


**Figure 2.** Myco 160 analysis of Mouse PD-L1 in CHO-K1/Mouse PD-L1 clone15 (Lane 09: M00567)

# Instruction for maintaining stable cell line

## Cell recovery

The cells were maintained in F-12K, 10% FBS, and 600 μg/ml GENETICIN *. The S.O.P for cell recovery is briefly introduced here:

1) Preheat a water bath to 37°C.
2) Remove the cryovial from the liquid nitrogen tank and thaw by gentle agitation in a 37°C water bath until ice crystals are melted, usually within 2-3 minutes.
3) Remove the vial from the water bath and decontaminate by spraying with 70% ethanol.
4) Unscrew the vial and transfer the cells to a 15 ml sterile conical centrifuge tube containing 9 ml complete growth medium.
5) After centrifugation at 125 g for 10 minutes, discard the supernatant and resuspend the cells in 2 ml of complete growth medium. Pipette gently to loosen the pellet and break apart clumps.
6) Transfer the cell suspension to the culture vessel with antibiotic free medium and mix thoroughly. Incubate cultures at 37°C, 5% $CO_2$.
7) Replace with fresh culture medium the next day (with appropriate concentration of antibiotic).

***** Concentration used for selection was 600 μg/ml GENETICIN.

## Cell Maintenance and Subculturing

Volumes are for 6 cm Dish proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

1) Bring the complete growth medium to 37°C in a water bath.
2) Remove and discard culture medium in the flask.
3) Briefly rinse the cell layer with Ca/Mg free DPBS to remove all traces of serum.
4) Add 1.0 to 2.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 10 minutes).

   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
5) Add 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
6) Centrifuge at 125 g for 10 minutes, discard the supernatant and resuspend the cells in 5 ml complete growth

medium.

7) Add appropriate aliquots of the cell suspension to new culture vessels. Incubate cultures at 37°C.

Subcultivation Ratio: 1:3 to 1:4

Medium Renewal: 2-3 times per week

## Cell Cryopreservation

1) Prepare a freeze medium consisting of complete growth medium and 5% DMSO.
2) Harvest cells by gentle centrifugation at 125 g for 10 minutes and resuspend them in the freeze medium at a concentration of 1 x $10^6$ to 5 x $10^6$ viable cells/ml. Continue to culture the cells until the viability of the recovered cells is confirmed.
3) Label the cryovials with the name of the cell line, then add 1 ml of the cell suspension to each of the vials and seal.
4) Place the vials into a pre-cooled (4°C) controlled-rate freeze chamber and place the chamber in a -80°C freezer for at least 24 hours.
5) Quickly transfer the vials to a liquid nitrogen tank.
6) After 24 hours in liquid nitrogen, take one vial and recover the cells to determine the cell viability.

# Packing List

**Cell lines** (Shipping Condition: -80°C Dry Ice, Store at -196°C)

**Name**: CHO-K1/Mouse PD-L1 clone15
**Quantity**: 1x10^6cells/vial
**Lot No.**: B30251703
**Number of vial**: 2 vials
**Store at**: -196°C

**Certified by:** Jan sun **Date:** ***09/04/2017***

# Appendix

## 1. Target gene information

*mouse PD-L1, NM_021893.3;*

## 2. Antibodies used for FACS:

Primary antibody: PE anti-mouse CD274 (B7-H1,PD-L1) (Biolegend,cat#124307)


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