Certificate of Analysis

Cat. No.: M00533

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Tel: 1-732-885-9188

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Certificate of Analysis

Project Information

Order Number: M00533

Host Cell: CHO-K1

Target Gene: VISTA

Product Information

	Cell Name	Cell Culture Medium	Freezing Medium
Product	CHO-K1/VISTA	F-12K, 10% FBS, 8 μg/mL Puromycin	95% complete growth medium, 5% (V/V) DMSO

Materials

Materials	Company	Cat. No.
F-12K	Gibco	21127-022
Puromycin	Gibco	A1113803
FBS	BIOIND	04-001-1A
0.25% Trypsin-EDTA	Gibco	25200-072
DMSO	SIGMA	D2650
MycoAlert™ PLUS Mycoplasma Detection Kit	Lonza	LT07-703

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QC Results

Validation by Flow Cytometry

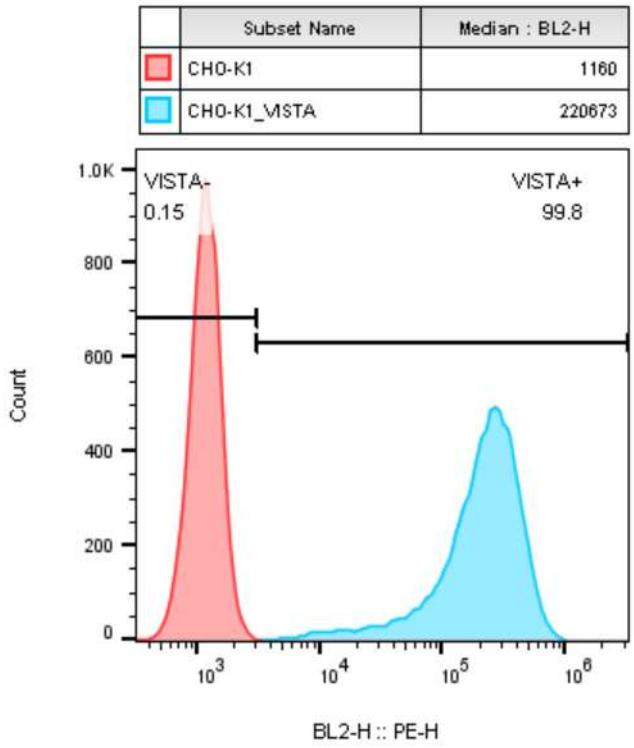

Figure 1. Flow cytometric analysis of CHO-K1/VISTA cells stained with anti-VISTA antibody.

Mycoplasma Testing

Cell	Value*	Result**
CHO-K1/VISTA	0.50	Passed

** The mycoplasma test was performed with MycoAlert™ PLUS Mycoplasma Detection Kit of Lonza. MycoAlert PLUS is able to detect almost all common Mollicutes contaminations with the exception of Ureaplasma. Ureaplasma utilizes a different metabolic pathway and lack the enzyme the kit will detect, but this isn't one of the organisms required by the FDA/USP and it would be highly unlikely to ever see this contamination in a cell culture.*

*** The test has been designed to give ratios of less than 1 with uninfected samples and routinely yield ratios greater than 1 for samples infected with mycoplasma.*

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Appendix A: Target Protein Information

Protein sequence of VISTA

MGVPTALEAGSWRWGSLLFALFLAASLGPVAAFKVATPYSLYVCPEGQNVTLTCRLLGPV
DKGHDVTFYKTWYRSSRGEVQTCSERRPIRNLTFQDLHLHHGGHQAANTSHDLAQRHGLE
SASDHHGNFSITMRNLTLLDSGLYCCLVVEIRHHHSEHRVHGAMELQVQTGKDAPSNCVV
YPSSSQDSENITAAALATGACIVGILCLPLILLLVYKQRQAASNRRAQELVRMDSNIQGI
ENPGFEASPPAQGIPEAKVRHPLSYVAQRQPSESGRHLLSEPSTPLSPPGPGDVFFPSLD
PVPDSPNFEVI

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Appendix B: Instruction for Maintaining Stable Cell Line

Cell Recovery

The standard operating procedure for cell recovery is briefly described here:

1) Prewarm a water bath to 37°C.
2) Remove the cryovial from the liquid nitrogen tank and thaw it 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 it by spray with 75% ethanol.
4) Unscrew the vial and transfer the cells to a 15-mL sterile conical centrifuge tube containing 9.0 mL complete growth medium (F-12K supplemented with 10% FBS).
5) After centrifugation at 100 × g for 4 minutes, discard the supernatant and resuspend the cell pellet in 2.0 mL complete growth medium. Pipette gently to loosen the pellet and break apart clumps.
6) Transfer the cell suspension to the culture vessel with complete growth medium and mix thoroughly. Incubate cultures at 37°C, 5% CO_2.
7) Aspirate the medium and replace with fresh culture medium (F-12K supplemented with 10% FBS and 8 μg/mL Puromycin) the next day.

Cell Maintenance and Subculturing

The cells were maintained in culture medium (F-12K supplemented with 10% FBS and 8 μg/mL Puromycin).

Volumes are for T-75 flask. Proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

1) Prewarm the culture medium to 37°C in a water bath.
2) Remove culture medium in the flask.
3) Briefly rinse the cell layer with Ca/Mg^{2+} free DPBS to remove the residual serum.
4) Add 2.0-3.0 mL of Trypsin-EDTA solution to the flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 2 to 5 minutes).
5) Add 8.0 mL culture medium and aspirate cells by gently pipetting.
6) Centrifuge at 100 × g for 4 minutes, discard the supernatant and resuspend the cell pellet in 5.0 mL culture medium.
7) Add appropriate aliquots of the cell suspension to new culture vessels. Incubate cultures

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at 37°C.

Subcultivation Ratio: 1:4 to 1:8 is recommended.

Medium Renewal: Every 2 to 3 days.

Cell Cryopreservation

1) Prepare a freezing medium consisting of complete growth medium and 5% DMSO.
2) Harvest cells by gentle centrifugation at 100 × g for 4 minutes and resuspend them in the freezing medium at a concentration of 1×10^6 viable cells/mL.
3) Label the cryovials with the name of the cell line, then add 1.0 mL of the cell suspension to each of the vials and seal the cap.
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.

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Appendix C: Packing List

Cell (Shipping Condition: Dry ice/Liquid nitrogen, Store at -196°C)

Name: CHO-K1/VISTA

Quantity: 1×10^6 cells/vial

Lot No.: B110011803

Frozen date: 03/28/2018

Number of vial: 2 vials

Store at: -196°C

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