Certificate of Analysis for NR-53522

Human Lung Carcinoma Cells (A549) Expressing Human Angiotensin-Converting Enzyme 2 (HA-FLAG)

Catalog No. NR-53522

Product Description:

NR-53522 contains a preparation of human *(Homo sapiens)* lung carcinoma epithelial cells (A549) expressing human angiotensin-converting enzyme 2 (ACE2) with C-terminal HA-tag and FLAG-tag. A549-hACE2 (HA-FLAG) cells were created by transducing A549 cells with lentiviral vector pLVX-Puro (Clontech™) expressing human ACE2 under the control of human cytomegalovirus immediate early promoter. pLVX-Puro contains a puromycin resistance gene for maintenance of stable transductants.

Lot: 70050562 **Manufacturing Date: 22FEB2022**

TEST	SPECIFICATIONS	RESULTS
Growth Properties	Adherent	Adherent
Morphology	Epithelial	Epithelial
Confirmation of ACE2 Expression by Immunofluorescence Assay (IFA)[1]	Fluorescence observed	Fluorescence observed (Figure 1)
Confirmation of HA-Tag Expression by IFA[2]	Fluorescence observed	Fluorescence observed (Figure 2)
Confirmation of FLAG-Tag Expression by IFA[3]	Fluorescence observed	Fluorescence observed (Figure 3)
Multiplex PCR Amplification of Cytochrome C Oxidase I (COI) Gene	Human origin No evidence of another species	Human origin No evidence of another species
Total Cell Count	> 1.0×10^6 cells per vial	4.8×10^6 cells per vial
Post-Freeze Viability	≥ 50%	86.3%
Sterility (21-day incubation)		
Harpo's HTYE broth, 37°C and 26°C, aerobic[4]	No growth	No growth
Trypticase Soy broth, 37°C and 26°C, aerobic	No growth	No growth
Sabouraud broth, 37°C and 26°C, aerobic	No growth	No growth
Sheep blood agar, 37°C, aerobic	No growth	No growth
Sheep blood agar, 37°C, anaerobic	No growth	No growth
Thioglycollate broth, 37°C, anaerobic	No growth	No growth
DMEM with 10% FBS, 37°C, aerobic	No growth	No growth
Mycoplasma Contamination		
Hoechst DNA stain	None detected	None detected
Agar and broth culture (14-day incubation at 37°C)	None detected	None detected
DNA detection by PCR of extracted Test Article nucleic acid	None detected	None detected

[1]Seed material tested using ACE2 mouse monoclonal antibody (Proteintech®, 66699-1-Ig)
[2]Seed material tested using HA Tag mouse monoclonal antibody (2-2.2.14), DyLight® 488 (Invitrogen™, 26183-D488)
[3]Seed material tested using DYKDDDDK Tag mouse monoclonal antibody (FG4R), DyLight® 488 (Invitrogen™, MA1-91878-D488)
[4]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

BEI Resources
www.beiresources.org
E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898

Figure 1: IFA for Confirmation of ACE2 Expression
(Seed material)

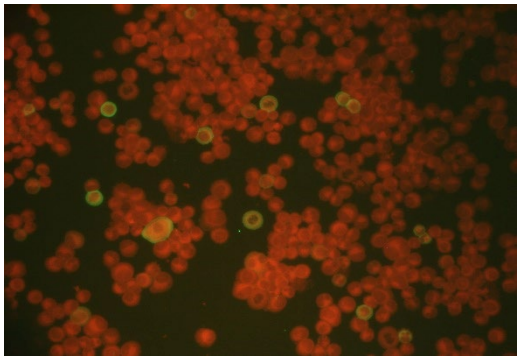


Figure 2: IFA for Confirmation of HA-Tag Expression
(Seed material)

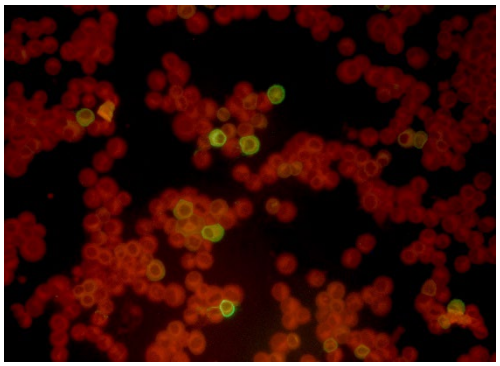
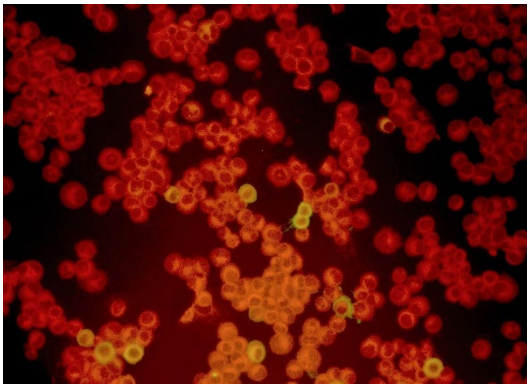


Figure 3: IFA for Confirmation of FLAG-Tag Expression
(Seed material)



/Sonia Bjorum Brower/
Sonia Bjorum Brower 10 JUN 2022
Lead Technical Writer or designee, ATCC Federal Solutions

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www.beiresources.org

E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898