b|e|i RESOURCES
SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-472

Enterovirus 71 (EV-71), MP4

Catalog No. NR-472

Product Description:

Cell lysate and supernatant from African green monkey (Vero) cells infected with EV-71, MP4.[1]

Lot: 7750290[2] **Manufacturing Date: 06DEC2006**

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero Cells[1]	Report results	Cell rounding and detachment
Identification by Indirect Fluorescent Antibody Assay		
Monoclonal antibody to EV-71[3]	Fluorescence observed	Fluorescence observed
Sequencing of a Strain-Specific Region (~ 580 nucleotides)	Report results	Identical to GenBank AF304458
RT-PCR Amplification of an Enterovirus-Specific Region	~ 680 base pair amplicon	~ 680 base pair amplicon
Titer by $TCID_{50}$ Assay in Vero Cells[1,4,5]	Report results	1.6 x 10^6 $TCID_{50}$/mL
Sterility (21-day incubation)		
Harpo's HTYE broth, 37°C and 26°C, aerobic[6]	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 and 5% CO_2	No growth	No growth
Mycoplasma Contamination		
Agar and broth culture (14-day incubation at 37°C)	None detected	None detected
DNA Detection by PCR of Test Article nucleic acid	None detected	None detected

[1]Vero cells: ATCC® CCL-81™ (lot 5067004)
[2]EV-71, MP4 was deposited by National Cheng Kung University, Tainan, Taiwan. NR-472 was grown from the deposited virus seed in Minimum Essential Medium containing Earle's salts and non-essential amino acids (Invitrogen™ 10370) supplemented with 2% irradiated fetal bovine serum (Cambrex® 14-471F), 2 mM L-glutamine (Invitrogen™ 25030) and 1 mM sodium pyruvate (Invitrogen™ 11360) for 3 days at 37°C and 5% CO_2.
[3]Millipore 3323
[4]The Tissue Culture Infectious Dose 50% ($TCID_{50}$) endpoint is the 50% infectious endpoint in cell culture. The $TCID_{50}$ is the dilution of virus that under the conditions of the assay can be expected to infect 50% of the culture vessels inoculated, just as a Lethal Dose 50% (LD_{50}) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the $TCID_{50}$ provides a measure of the titer (or infectivity) of a virus preparation.
[5]6 days at 34°C and 5% CO_2
[6]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

/Heather Couch/
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