

Certificate of Analysis for NR-51845

Enterovirus 71, Tainan/4643/1998, Mouse-Adapted

Catalog No. NR-51845

Product Description:

Enterovirus 71 (EV-71), Tainan/4643/1998, mouse-adapted was derived from EV-71, Tainan/4643/1998 which had undergone four serial passages in mice (BEI Resources NR-472). The virus was further adapted by six sequential passages in AG129 mice followed by plaque purification in rhabdomyosarcoma cells.

Passage History:

M(10)RD(7)/RD(2) (Prior to deposit at BEI Resources/BEI Resources); M = Mice; RD = Rhabdomyosarcoma cells¹

Lot: 70029769² Manufacturing Date: 25OCT2019

| TEST | SPECIFICATIONS | RESULTS |
|---|---|---|
| Identification by Infectivity in RD cells ¹ | Cell rounding and detachment | Cell rounding and detachment |
| Sequencing of Species-Specific Region (~ 870 nucleotides) | ≥ 98% identity with EV-71, Tainan/4643/1998 (GenBank: AF304458.1) | 99.5% identity with EV-71, Tainan/4643/1998 (GenBank: AF304458.1) |
| Titer by TCID ₅₀ Assay in RD cells by Indirect Fluorescent Antibody (IFA) Assay ^{1,3,4,5} | Report results | 8.9 × 10 ⁸ TCID ₅₀ per mL |
| Amplification of EV71 Sequence by RT-PCR | ~ 890 base pair amplicon | ~ 890 base pair amplicon |
| 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 |
| Blood agar, 37°C, aerobic | No growth | No growth |
| 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 extracted Test Article nucleic acid | None detected | None detected |

¹Rhabdomyosarcoma cells (RD; ATCC[®] CCL-136™)

/Heather Couch/ Heather Couch

20 JAN 2020

Program Manager or designee, ATCC Federal Solutions

BEI Resources www.beiresources.org E-mail: contact@beiresources.org

Tel: 800-359-7370 Fax: 703-365-2898

²Grown in Eagle's Minimum Essential Medium containing Earle's Balanced Salt Solution, non-essential amino acids, 2 mM L-glutamine, 1 mM sodium pyruvate and 1.5 g/L of sodium bicarbonate (ATCC® 30-2003) supplemented with 2% fetal bovine serum (ATCC® 30-2020) for 3 days at 37°C with 5% CO₂.

³The Tissue Culture Infectious Dose 50% (TCID₅₀) endpoint is the 50% infectious endpoint in cell culture. The TCID₅₀ 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% (LD₅₀) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the TCID₅₀ provides a measure of the titer (or infectivity) of a virus preparation. ⁴Assay plates were incubated 7 days at 37°C with 5% CO₂

⁵Detected using Pan-Enterovirus Reagent (Light Diagnostics™ 3360)

⁶Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.



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E-mail: contact@beiresources.org

Tel: 800-359-7370 Fax: 703-365-2898