

Toxoplasma gondii, Strain EGS SAG1-mCherry LDH2-sfGFP MSF-BFP

Catalog No. NR-53931

Product Description:

Toxoplasma gondii (*T. gondii*), strain EGS SAG1-mCherry LDH2-sfGFP MSF-BFP was deposited to BEI Resources as a transgenic strain that expresses the red fluorescent protein mCherry, superfolder green fluorescent protein (sfGFP) and blue fluorescent protein (BFP) at specific stages of the parasite's life cycle. Strain EGS SAG1-mCherry LDH2-sfGFP MSF-BFP was derived from the recombinant type I/III strain EGS, isolated in 1998 from amniotic fluid of a human patient with congenital toxoplasmosis in Brazil, that is highly virulent in mice and is able to spontaneously form cysts *in vitro*. NR-53931 was produced by cultivation of the deposited material in human foreskin fibroblast cells (ATCC® CRL-1634™) in Dulbecco's Minimal Essential Medium (DMEM), adjusted to contain 10% (v/v) heat-inactivated fetal bovine serum (HIFBS) for 10 days at 37°C in an aerobic atmosphere with 5% CO₂ until lysis of the host cell monolayer was reached.

Lot: 70043679

Manufacturing Date: 24MAY2021

TEST	SPECIFICATIONS	RESULTS
Cell Morphology¹ 6 days at 37°C in an aerobic atmosphere with 5% CO ₂ in DMEM supplemented with 10% HIFBS in human foreskin fibroblast cells (ATCC® CRL-1634™)	Report result	Refractive; crescent-shaped tachyzoites visible
Genotypic Analysis² Sequencing of uracil phosphoribosyltransferase (UPRT) intron 1 (~ 500 base pairs)	Consistent with <i>T. gondii</i> ≥ 99% sequence identity to <i>T. gondii</i> , strain EGS (BEI Resources NR-44106)	Consistent with <i>T. gondii</i> (Figure 1) 100% sequence identity to <i>T. gondii</i> , strain EGS (BEI Resources NR-44106)
Phenotypic Analysis^{2,3} Green fluorescent protein (sfGFP) expression mCherry red fluorescent expression	Positive Positive	Positive (Figure 2) Positive (Figure 2)
Viable Cell Count by Hemacytometry¹	> 10 ⁶ cells per mL	1.9 × 10 ⁷ cells per mL
Viability¹ 6 days at 37°C in an aerobic atmosphere with 5% CO ₂ in DMEM supplemented with 10% HIFBS in human foreskin fibroblast cells (ATCC® CRL-1634™)	Growth	Growth
Sterility (21-day incubation)¹ Harpo's HTYE broth, 37°C and 26°C, aerobic ⁴ Trypticase soy broth, 37°C and 26°C, aerobic Sabouraud broth, 37°C and 26°C, aerobic DMEM with 10% FBS, 37°C, aerobic Sheep blood agar, 37°C, aerobic Sheep blood agar, 37°C, anaerobic Thioglycollate broth, 37°C, anaerobic	No growth No growth No growth No growth No growth No growth No growth	No growth No growth No growth No growth No growth No growth No growth
Mycoplasma Contamination DNA Detection by PCR	None detected	None detected

¹Testing completed on vial, post-freeze material.

²Testing completed on bulk material prior to vialing and freezing.

³mCherry and sfGFP expression were examined by fluorescence microscopy in *T. gondii*-infected human foreskin fibroblast cells (ATCC® CRL-1634™). Approximately 25% of infected cells displayed tachyzoites with red fluorescence. Expression of BFP was not determined.

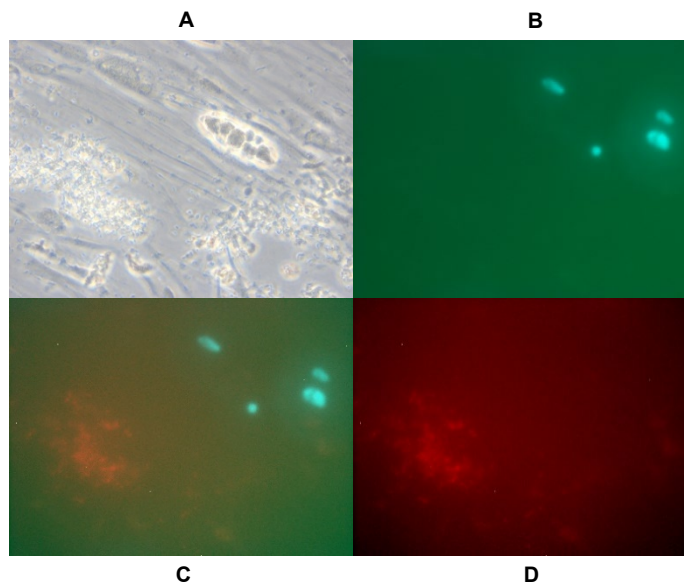
⁴Atlas, Ronald M. *Handbook of Microbiological Media*. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Figure 1: *T. gondii*, Strain EGS SAG1-mCherry LDH2-sfGFP MSF-BFP – UPRT Intron 1 Sequence

```

AAACGACCAG GAAGAAAGCA TTCTCCAGGA CATCATCACG AGGTAATCCT TCAACCGAAG TTTGCTTTCC GTGACTCTGC CTCTTGGTTA
TACTGCGTGG CCTTCCCGTC CTGCGGCCCC CTTTCTCCG CTTGCTGTTT AAATGCTCGT CCTCGTTTTT CTTCCTGCCG CATCCCCGTA
TATTTTAAGG AGAGGGAAAC AGGCGTGAGT TGGACGGAAT GAAAGTTCTC GGCCTGTACG CCGGTTGTCG CCGTCTGTTG CAGATTGCTT
TTTTCTTCGA ATCGGTGCTG TAACCTCGA AGAAGAACA CGCTGCAAAC GACTTGTCTG ACTCTCAGTC GTGTACTTTA CGTGCTTCCT
TTCAGGGACC TCCCTCCGCG TTACTCATT GTATTACAG CTACGAAGT TCTTGAAGG TGGATTCTGT CCAGGCTCCA TGTCTCACTC
GGTGCGTTTT CGGAAAAGTT CATTGTGAAC GTTCCCCTTG CGTGTATGA CTTTATCAGG TTTCCCAATG TGGTGCTCAT
    
```

Figure 2: Phenotypic Analysis



- 2A:** *T. gondii*-infected human foreskin fibroblast cells (ATCC® CRL-1634™) under bright light.
- 2B:** Visualization of GFP-expressing *T. gondii* bradyzoites under green fluorescent light.
- 2C:** Visualization of mCherry-expressing *T. gondii* tachyzoites under red fluorescent light.
- 2D:** Merged images 2B and 2C showing mCherry-expressing *T. gondii* tachyzoites and GFP-expressing bradyzoites in the same field of view.

/Heather Couch/
Heather Couch

Program Manager or designee, ATCC Federal Solutions

12 OCT 2021

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.

ATCC® is a trademark of the American Type Culture Collection.
You are authorized to use this product for research use only. It is not intended for human use.

