pSVA3

77273™

Description

This clone encodes the L22F mutant of DHFR that can serve as a dominant selection marker because it retains catalytic activity and also confers methotrexate (MTX) resistance at low MTX concentrations (125 nM). Transfected cells amplify DHFR sequences. Constructed by cloning a HindIII/SmaI fragment containing the gene into pSV2-neo from which neoR had been removed. This plasmid was then cut with SmaI and BglII to remove 18 G nucleotides at the 3? end. The BglII site was filled in, and ligated to the SmaI site, thereby destroying the original BglII and SmaI sites. Removing the HindIII/SmaI fragment from the pSV2-neo removes all but the 3? 171 bp of neoR.

Organism: *Cricetulus griseus*, hamster, Chinese
Clone type: Clone
Host: *Escherichia coli* HB101 (ATCC 33694)

Storage Conditions

Product format: Freeze-dried
Storage conditions: 2°C to 8°C

Intended Use

This product is intended for laboratory research use only. It is not intended for any animal or human therapeutic use, any human or animal consumption, or any diagnostic use.

BSL 1

ATCC determines the biosafety level of a material based on our risk assessment as guided by the current edition of *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, U.S. Department of Health and Human Services. It is your responsibility to understand the hazards associated with the material per your organization's policies and procedures as well as any other applicable regulations as enforced by your local or national agencies.

Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at www.atcc.org.

Insert Information

Insert size (kb): 0.70999999999999996
Type of DNA: cDNA
Insert source: lung cell line
Insert tissue: lung cell line
Insert information:
DESCRIPTION OF INSERT COMPONENT:
Insert 5' end: HindIII
Insert 3' end: BglII
Cross references:
Genome: hamster, Chinese
Target gene: dihydrofolate reductase
Gene name: dihydrofolate reductase
Gene product: dihydrofolate reductase [DHFR]
Gene symbol: DHFR
Contains complete coding sequence: Unknown

Vector Information

Construct size (kb): 5.099999904632568
Intact vector size: 5.729
Vector name: pSV2-neo
Type of vector: plasmid
Construction: SV40, Tn5, pBR322
Host range: vertebrate cells
Vector end: HindIII; SmaI
Vector information:
Cross references: DNA Seq. Acc.: U02434
Cloning sites: EcoRI; BamHI; PvuII; PstI; HindIII
Markers: ampR
Promoters: SV40 early
Replicon: SV40; pMB1
Restriction sites: HindIII

Growth Conditions

Medium:
ATCC Medium 1227: LB Medium (ATCC medium 1065) with 50 mcg/ml ampicillin
Temperature: 37°C

Handling Procedures

Transfer a loopful to a test tube containing 5 mL LB+50mg/mL of ampicillin broth. A loopful of culture can also be streaked on an LB + amp agar plate. Incubate cultures at 37°C. Isolate DNA using standard plasmid preparation procedures.

Notes

Restriction digests of the clone gave the following sizes (in kb): HindIII/SmaI -- 5.2; BamHI -- 3.5, 1.7 ; EcoRI -- 2.7, 2.3 ; PstI -- 1.9, 1.4, 0.95, 0.9 ; PvuII -- 4.0, 1.1.

---ATCC staff

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- ATCC staff

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- Gene 112: 179-188, 1992

Transfected cells amplify DHFR sequences.
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Constructed by cloning a HindIII/SmaI fragment containing the gene into pSV2-neo from which neoR had been removed. This plasmid was then cut with SmaI and BglII to remove 18 G nucleotides at the 3' end.
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The BglII site was filled in, and ligated to the SmaI site, thereby destroying the original BglII and SmaI sites.
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Removing the HindIII/SmaI fragment from pSV2-neo removes all but the 3' 171 bp of neoR.
- Gene 112: 179-188, 1992

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: pSVA3 (ATCC 77273)

References

References and other information relating to this material are available at www.atcc.org.

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