

CopyControl™ Fosmid Autoinduction Solution

Cat. No. AIS107F

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1. Introduction

The CopyControl™ Fosmid Autoinduction Solution is designed to induce CopyControl Fosmid clones and clones retrofitted with the EZ-Tn5™ <oriV/KAN-2> Transposon, grown in TransforMax™ EPI300™ *E. coli* cells, from single-copy number to a higher-copy number of approximately 50 fosmids per cell.

The Fosmid Autoinduction Solution induces expression of a mutant *trfA* gene contained in the TransforMax EPI300 cells. Expression of *trfA* gene results in initiation of replication from the *oriV* high copy origin of replication and subsequent amplification of the CopyControl clones and clones containing the EZ-Tn5 <oriV/KAN-2> Transposon to high copy number.

The new Fosmid Autoinduction protocol improves upon the existing induction protocol by including the autoinduction supplement in the media prior to culture inoculation, removing the need for time-consuming subculturing and the 2-hour incubation required in the standard induction protocol. The Fosmid Autoinduction Solution also contains cell growth enhancers which boost cell numbers and typically provides higher DNA yields than with the standard CopyControl induction protocol.

The hands-off autoinduction protocol makes CopyControl Fosmid Autoinduction solution ideal for high-throughput purification protocols in 96-well format. The autoinduction solution is also compatible with larger scale DNA purifications and can be scaled according to the amount of media used.

2. Product Specifications

Storage: Store only at –20°C in a freezer without a defrost cycle. Mix thoroughly after thawing.

Size and Formulation: CopyControl Fosmid Autoinduction Solution is available in a 50-ml size at an approximately concentrate of 500X in sterile water. This is sufficient to supplement 25 liters of growth media.

Quality Control: CopyControl Fosmid Autoinduction Solution is function-tested to induce CopyControl Fosmid clones from single-copy number to high-copy number at a 1X final concentration.

3. Related Products

The following products are also available:

- CopyControl™ BAC Cloning Kits
- CopyControl™ cDNA, Gene & PCR Cloning Kits
- CopyControl™ Fosmid Library Production Kit
- EZ-Tn5™ <oriV/KAN-2> Insertion Kit
- TransforMax™ EPI300™ *E. coli*
- CopyControl™ BAC Autoinduction Solution
- CopyControl™ Induction Solution

4. Autoinduction of CopyControl Fosmid Clones and Fosmid Clones Retrofitted with an EZ-Tn5 <oriV/KAN-2> Transposon to High-Copy Number

CopyControl Fosmid clones and low-copy number fosmid clones retrofitted with an EZ-Tn5 <oriV/KAN-2> Transposon and grown in TransforMax EPI300 cells can be amplified to ~50 copies per cell. Generally, 1.2 ml of an autoinduced culture will provide a sufficient amount of fosmid DNA for most applications including end sequencing and fingerprinting.

Autoinduction protocols in 96-well plates are typically performed in 1.2 ml of culture in a 2-ml growth plate. Larger cultures can be autoinduced as well, volumes can be scaled up as required by the user. Proper aeration is critical to the success of the autoinduction process.

Important: *The Growth Media for amplifying CopyControl Fosmid clones and low-copy number fosmid clones retrofitted with an EZ-Tn5 <oriV/KAN-2> Transposon are different. Be sure to use the appropriate Growth Media for the type of fosmid clones that you are amplifying.*

Growth Media for CopyControl Fosmid Clones

LB + chloramphenicol (12.5 µg/ml) + Fosmid Autoinduction Solution (2 µl/ml)

Growth Media for Fosmid Clones Retrofitted with an EZ-Tn5 <oriV/KAN-2> Transposon

LB + chloramphenicol* (12.5 µg/ml) + kanamycin (50 µg/ml) + Fosmid Autoinduction Solution (2 µl/ml)

*or other selectable marker present on the cloning vector backbone

Growth Conditions: Incubate cultures in an incubator/air shaker for approximately 17 hours at 37°C with constant shaking at 250 rpm.

Notes:

1. Cultures that are grown for more than 20 hours could suffer from clone stability or toxicity problems. Extended incubations should be avoided.
2. Cultures grown less than 15 hours may not be fully induced and could produce lower than desired DNA yields.
3. The amount of CopyControl Fosmid Autoinduction Solution added and the amount of growth media used can be freely scaled to match the desired yields. Unlike induction with the CopyControl Induction Solution, growth conditions in 96-well format and large flask cultures are identical.
4. Autoinduction is only effective on Epicentre's CopyControl Fosmids and fosmids fitted with the EZ-Tn5 <oriV/KAN-2> transposon grown in Epicentre's TransforMax EPI300 cells. Standard single-copy fosmids or CopyControl fosmids in other cell lines are not inducible with CopyControl Fosmid Autoinduction Solution.

5. CopyControl BACs require a different Autoinduction Solution (CopyControl BAC Autoinduction Solution, Cat. No. AIS107B). The BAC and Fosmid Autoinduction solutions are not interchangeable and will produce sub-optimal results if used with the improper vector type.

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