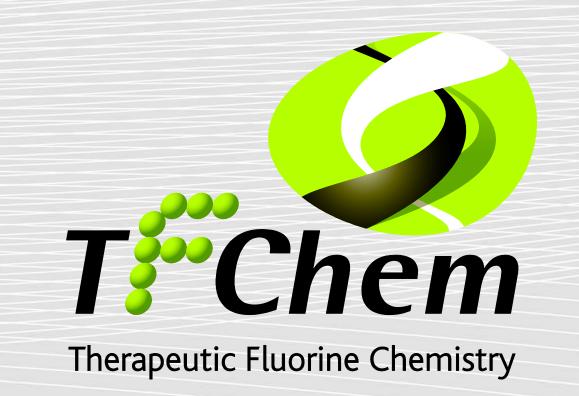


Order IPGMimTM

A New, Improved Inducer for Difficult to Express Proteins



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BACKGROUND

Sirona Biochem specializes in carbohydrate chemistry. Its subsidiary TFChem applies a proprietary process that mimics and stabilizes compounds with the aim of producing inducers that initiate protein expression more efficiently than currently marketed inducers. This chemistry technique has been applied to produce IPGMim™ (TFC-358), a new inducer that can improve the production and product margins of recombinant proteins (Patent EP11306021.4).

With increased use of recombinant proteins in therapeutic applications and other industries, there is a recognized need for a more stable inducer that improves the quality of expression and provides better control of protein expression, particularly over long periods of induction. The quality and quantity of protein expressed are related to the type of inducer, its concentration, the speed and duration of the induction, and the conditions applied to the expression system.

Isopropyl β-D-1-thiogalactopyranoside (IPTG) is the most common biological reagent and is the most widely used to induce the production of recombinant proteins in Escherichia coli. IPTG's degrading nature results in a number of limitations such as storage and handling conditions. IPTG and its solutions are ideally stored at -20°C requiring appropriate on-site storage at production facilities. Furthermore, IPTG cannot maintain induction for long periods and a minimum concentration of IPTG is required for it to be efficient.

A fast induction does not work for all proteins and can lead to suboptimal yields. For some recalcitrant proteins (e.g. weakly expressed or insoluble), a slow induction is preferred to enhance the yield of the protein.

The production of foreign protein in a host cell often exerts upon the

cells a severe metabolic load that affects their normal function. This leads to a dramatic loss in cell viability, reducing the product formation window and the overall yield. Therefore, to take full advantage of the capabilities of the host/vector system, the recombinant gene expression has to be modulated. Transcription rate modulation can be attained by acting on the supply of inducer. (Biotechnol. Prog. 2008, 24, 667-674)

When lowering the concentration of IPTG to achieve slower induction, the compound undergoes degradation thereby further reducing concentration to a level that ceases induction.

IPGMim[™] has been evaluated to demonstrate its potency in controlling the transcription rate and improving production yield. IPGMim™ is a stable inducer at room temperature and does not require any special storage conditions.

HOW IPGMim™ WORKS

DISADVANTAGES OF IPTG

IPGMim™ is a new inducer of recombinant protein expression controlled by the *lac operon*. **IPGMim™** is a mimic of commonly used IPTG, which triggers transcription of the lac operon by binding to the lac repressor and allowing the transcription of genes (e.g. beta-galactosidase)

- UNSTABLE It must be stored at -20°C or -4°C It requires multiple additions in order to sustain the level of protein expression in experiments
- Some proteins remain difficult to express (low yield, low quality)

TESTING IPGMim™

VALUE ADDED RESULTS FOR IPGMim™

Sirona Biochem conducted a series of qualitative and quantitative tests for IPGMim™, using different proteins, parameters and conditions. Toxicity studies with E.coli, a commonly used agent in protein expression were also conducted. The tests were met with positive results. A number of companies/institutions also tested IPGmim with marked successes. Ten proteins were tested. Further tests will be conducted for the creation of a wider portfolio of recalcitrant recombinant proteins.

- Stability (no degradation at room temperature)
- > Same level of effect on *E.coli* growth than IPTG: no additional toxicity
- Longer duration of action
- > Better Induction at lower concentrations than IPTG (depending on the protein, it was observed between 2 and 56h of induction)
- > Increases the yield of the total fraction of some recombinant proteins at low concentration (<0.1mM)
- > Better inducer than IPTG for recombinant proteins that need slow and long induction (at least 24h) > Increases the yield of recalcitrant proteins where a slow but long induction is preferred.

CONCLUDING THE CASE FOR IPGMim™

