APPLICATION FOR THE APPROVAL OF 2'-FUCOSYLLACTOSE (2'-FL) FROM CORYNEBACTERIUM GLUTAMICUM UNDER THE AUSTRALIA AND NEW ZEALAND FOOD STANDARDS CODE

EXECUTIVE SUMMARY

PREPARED BY:

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Advanced Protein Technologies Corp. is submitting an application to Food Standards Australia New Zealand (FSANZ) concerning a purified human milk oligosaccharide (HMO) ingredient, 2'-fucosyllactose (2'-FL), produced by fermentation using a *Corynebacterium glutamicum* production strain. The applicant is seeking approval for the use of its 2'-FL ingredient in infant formula products as a nutritive substance. This product, 2'-FL, is comprised of not less than 94% 2'-FL as the primary constituent and lesser amounts of other carbohydrates such as difucosyllactose, glucose, and galactose. While 2'-FL from *C. glutamicum* meets the general purity specification parameters for 2'-fucosyllactose as currently defined in *Schedule 3 – Identity and purity* of the *Australia New Zealand Food Standards Code* ("the Code"), *C. glutamicum* is not listed as a permitted source organism for producing this ingredient.

The 2'-FL ingredient from *C. glutamicum*, similar to other 2'-FL preparations already permitted for use in infant formula in Australia and New Zealand, would be used as a nutritive substance for inclusion in infant formula products. 2'-FL from *C. glutamicum* would have the same approved uses (*i.e.*, infant formula products) and use levels (*i.e.*, 96 mg/kJ or 2.4 g/L) as other 2'-FL preparations already approved in Australia and New Zealand. The availability and use of 2'-FL from *C. glutamicum* as an alternative source for this HMO ingredient is expected to promote healthy market competition, which will ultimately benefit Australian and New Zealand consumers.

Production of the applicant's 2'-FL from *C. glutamicum* is conducted in accordance with current Good Manufacturing Practice and Hazard Analysis and Critical Control Points. The 2'-FL ingredient is produced by fermentation of glucose and lactose using a strain of *C. glutamicum*. During production, the 2'-FL is released into the fermentation medium, where it then undergoes a series of filtration and purification steps before a final downstream processing step (either crystallisation or spray drying). Upon completion of the production process, the 2'-FL ingredient does not contain any of the production organism and is then packaged as the final ingredient. The final HMO product, 2'-FL, is a high-purity ingredient that contains no less than 94% 2'-FL.

Product specifications for 2'-FL from *C. glutamicum* have been established based on the Generally Recognized as Safe (GRAS) ingredient marketed in the United States (U.S.). The specifications present relevant purity parameters, as well as limits for heavy metals and microbiological parameters. The established specifications also align with the specifications for 2'-FL from a genetically modified strain of *C. glutamicum* ATCC 13032 permitted for use in the European Union, as well as the approved 2'-FL preparations produced using other microbial sources currently included in *Schedule 3 – Identity and purity* of the Code. Batch analysis of 10 non-consecutive lots of 2'-FL from *C. glutamicum* demonstrate that the applicant's manufacturing process produces a consistent product that meets the defined specifications. Additionally, the absence of viable cells or residual DNA from the production strain has been confirmed in 5 commercial lots of 2'-FL using a targeted culture-based method to detect viable cells and 10 different quantitative polymerase chain reaction methods to detect residual DNA.

2'-FL has a long history of established safe consumption in the human diet as a significant component in the HMO fraction of human breast milk. Analytical data demonstrate that 2'-FL from *C. glutamicum* is chemically identical to the 2'-FL naturally present in human breast milk and can therefore be expected to undergo the same metabolic pathways. In brief, 2'-FL is a non-digestible oligosaccharide that passes through the upper gastrointestinal tract intact and is digested by anaerobic bacteria of the colon or excreted in the faeces. A series of toxicological studies has been conducted with 2'-FL from *C. glutamicum*, including a bacterial reverse mutation assay, an *in vitro* chromosomal aberration test in Chinese hamster lung cells, an *in vivo* micronucleus test in rats, and oral acute and subchronic toxicity studies in rats. The safety of 2'-FL from both chemical synthesis and microbial fermentation has also been extensively evaluated by several scientific bodies and regulatory agencies. For example, 2'-FL from *C. glutamicum* APC199 has been evaluated by the European Food Safety Authority and the U.S. Food and Drug Administration (FDA), while other 2'-FL ingredients have been evaluated by Health Canada, the Ministry of Health of Israel, and also, FSANZ. More specifically, 2'-FL from *C. glutamicum* APC199 has GRAS status for use as a food ingredient in a variety of

food and beverage applications. The FDA reviewed GRAS Notice 000932 describing the production of 2'-FL from *C. glutamicum* and responded on 26 February 2021 with a "no questions" letter regarding the GRAS status of 2'-FL.[1] The data evaluated in the various reviews by scientific bodies and regulatory agencies are summarised in the accompanying application and include an extensive range of 2'-FL studies of *in vitro* and *in vivo* mutagenicity and genotoxicity, short- and long-term toxicity and developmental toxicology studies in rats and pigs, and safety studies conducted in humans. A comprehensive search of the published scientific literature was conducted to identify relevant safety studies on 2'-FL that were not evaluated in the most recently gazetted FSANZ application for 2'-FL produced by microbial fermentation (*i.e.*, A1233). The studies identified in the literature search did not change the overall conclusion of safety that has been established for other approved 2'-FL preparations.

Since 2'-FL from fermentation is produced using a microbial production strain, the safety of the production microorganism (*C. glutamicum*) was also considered in this application. *C. glutamicum* is a non-pathogenic bacterium widely used in industrial amino acid production and has been studied for more than 60 years since its discovery as a glutamate-secreting bacterium. In the European Union, *C. glutamicum* has been granted qualified presumption of safety status for the production of amino acids and other food ingredients such as flavouring compounds. While 2'-FL from *C. glutamicum* has been thoroughly analysed to demonstrate an absence of residual protein and viable cells in the final ingredient, a series of bioinformatic searches were conducted to evaluate the potential allergenicity of proteins encoded by genes required for 2'-FL biosynthesis and to evaluate the potential toxigenicity of the whole *C. glutamicum* APC199 genome sequence. No evidence of allergenicity or toxigenicity potential was identified in any of the sequence alignment searches.

2'-FL from *C. glutamicum* is intended for use as a nutritive substance in Australia and New Zealand under the same conditions of use as those presently authorised for other 2'-FL preparations in infant formula products. As such, the use of 2'-FL from *C. glutamicum* is expected to be fully substitutional to the 2'-FL preparations currently marketed in Australia and New Zealand, and the anticipated intakes of this ingredient are unlikely to change from the current levels in the result of a successful application. A separate intake assessment for 2'-FL from *C. glutamicum* therefore was not performed for this application. Additionally, since FSANZ previously assessed 2'-FL from multiple sources as an ingredient in infant formula products, and the current application relates only to a new source of 2'-FL without proposing extensions in use or different use levels, information related to the nutritional impact of 2'-FL and its impact on consumer understanding and behaviour is not required.

The totality of evidence provided in this application supports the safe use of 2'-FL from *C. glutamicum* as a nutritive substance in infant formula products in Australia and New Zealand. 2'-FL is produced by fermentation of simple sugars (glucose and lactose) using a *C. glutamicum* production strain and is a high-purity HMO product containing no less than 94% 2'-FL. The applicant's 2'-FL has been analytically demonstrated to be chemically and structurally identical to 2'-FL produced synthetically and naturally present in human breast milk. Moreover, a growing body of research is available to indicate that 2'-FL is identical regardless of the production organism, and the final 2'-FL products are therefore considered substantially equivalent. The extensive safety database that exists for 2'-FL from fermentation with other production organisms can therefore be applied in conjunction with product-specific data to establish the safety of 2'-FL from *C. glutamicum*. The weight of the scientific evidence presented in the accompanying application indicates that consumption of 2'-FL from *C. glutamicum*, through its use as a nutritive substance in infant formula products, does not present a significant risk to human health and is safe for the intended use.

[1] U.S. FDA (2021). *Agency Response Letter GRAS Notice No. GRN 932 [2'-Fucosyllactose, Hwasung City, Gyeonggi-do, Republic of Korea: Advanced Protein Technologies Corp]*. Silver Spring (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=932 [Feb. 18, 2021 - FDA response - no questions].