Executive Summary

This application seeks regulatory approval for the food use of genetically modified bread wheat (*Triticum aestivum* L.) event UM-12115T-1. The event was developed via *Agrobacterium tumefaciens*-mediated transformation of the Australian wheat cultivar 'Gladius' to enhance grain nutrient density. This nutritional enhancement is achieved through constitutive expression of the *OsNAS2* (Nicotianamine Synthase 2) gene derived from rice (*Oryza sativa*). The constitutive expression of *OsNAS2* increases the biosynthesis of nicotianamine (NA), a naturally occurring plant chelator essential for the uptake, long-distance transport, and grain loading of transition metals such as iron and zinc.

A comprehensive molecular characterisation utilising PacBio HiFi long-read sequencing confirmed the precise genomic architecture of the modification. The transformation resulted in two independent, stable insertion loci located on Chromosome 1D (Locus 1) and Chromosome 4D (Locus 2). Because these loci are located on separate chromosomes, they are not genetically linked. Subsequent introgression of the trait into elite wheat varieties via conventional breeding will result in independent segregation, yielding three distinct genetic configurations: lines containing both Locus 1 and Locus 2, lines containing only Locus 1, and lines containing only Locus 2. This application encompasses all three variants. Both loci contain a single copy of the intended transfer-DNA expression cassettes. While Locus 2 is a simple insertion, Locus 1 contains a discrete, rearranged fragment of functionally inert vector backbone resulting from a complex integration event. The structural integrity of both insertion loci is fixed and has been demonstrated to be stably inherited through eight generations of self-pollination from T0 to T8. Furthermore, an extensive bioinformatic assessment of 94 putative open reading frames spanning the integration junctions and backbone sequences confirmed the absence of biologically relevant sequence homology to known allergens or protein toxins.

Wheat event UM-12115T-1 contains two introduced proteins: the intended trait protein, OsNAS2, hygromycin phosphotransferase (HPT) a selectable marker used during plant transformation. In addition, Locus 1 contains an unintended, non-functional fragment of the vector backbone comprising the Neomycin-kanamycin phosphotransferase (Kan) binary vector selectable marker. Liquid Chromatography-Parallel Reaction Monitoring-Mass Spectrometry demonstrated that the OsNAS2 protein is present at very low levels in edible flour, with a mean concentration of 0.024 ng/mg of extractable protein. Conversely, the HPT and Kan selectable marker proteins were not detectable in either the grain or leaf tissues, confirming the Kan backbone sequence is transcriptionally and translationally inert *in planta*.

OsNAS2, HPT and Kan do not share sequence homology with known allergens or toxins, and none of the proteins are predicted to enter the secretory pathway, thereby precluding host-specific unexpected post-translational modifications such as glycosylation. The proteins are highly susceptible to proteolytic degradation. Direct *in vitro* assays demonstrated that OsNAS2 is completely degraded within 30 seconds of exposure to simulated gastric fluid containing pepsin. Additionally, OsNAS2 is thermolabile and is rapidly inactivated at standard food processing temperatures. Deterministic dietary exposure assessments for both human populations in Australia and New Zealand, as well as for livestock, confirm that potential exposure to the OsNAS2 protein is exceptionally low, strictly remaining in the low nanogram per kilogram of body weight per day range.

A rigorous multi-environment compositional assessment was conducted across multiple locations and growing seasons to establish the metabolic and nutritional equivalence of UM-12115T-1 against a near-isogenic wild type control and commercial reference varieties. The combined-site analysis confirmed that UM-12115T-1 grain is strictly equivalent to conventional wheat in all key proximates, dietary fibre, amino acid profiles, fatty acid composition, and estimated metabolizable energy. The genetic modification does not disrupt secondary metabolism or elevate anti-nutritional profiles, with phytic acid levels remaining comparable to those of conventional varieties. Furthermore, no novel accumulation of toxic heavy metals was detected. The intended downstream metabolic effect of elevated NA concentration was consistently expressed and reliably maintained across diverse agronomic environments. These elevated NA levels in UM-12115T-1 remain well within the natural physiological ranges already established by conventional high-NA wheat germplasm.

The comprehensive molecular, biochemical, and compositional data presented in this application definitively demonstrate that wheat event UM-12115T-1 is structurally stable, safe for consumption, and compositionally equivalent to traditional commercial wheat. The event introduces no novel toxicological, allergenic, or nutritional hazards and will interact with the food supply chain in a manner fundamentally identical to conventional *Triticum aestivum*.