AMCAP™: Effortless mRNA Manufacturing by Next-Generation One-Pot Enzymatic Reaction

Massimo Carraro1 , Cristina Chiriaco1 , Maura Guardabascio1 , Marisa Marzocca1 , Luca Frattini1 , Chiara Crosta1 , Marco Pupo1 1Anemocyte s.r.l., Via Roberto Lepetit, 34, 21040 Gerenzano VA, Italy Background: The expansion of mRNA-based therapeutics highlights the critical need for scalable, efficient, and cost-effective manufacturing processes. Traditional mRNA production heavily relies on either a two-step enzymatic capping process or standard co-transcriptional methods. The former involves multiple purifications steps, which increase processing time and lower overall efficiency, while the latter lacks overall flexibility and accessibility. To overcome these limitations, Anemocyte presents AMCAP™, a proprietary, patent-pending one-pot technology designed to seamlessly combine in vitro transcription (IVT) and enzymatic capping within a single reaction. Method: The AMCAP™ technology was evaluated against standard two-step enzymatic capping and co-transcriptional methods. Essential parameters were analyzed, including mRNA production yield, capping efficiency via high-resolution mass spectrometry and enzymatic-based assays, and double-stranded RNA (dsRNA) contaminants. Process flexibility was assessed using several commercially available reagent mixes and two different transcription initiators (GGG and AGG). Functional validation was performed through in vitro protein expression assays across diverse targets, including EGFP, Luciferase, Cas9, mCherry, Cre recombinase and β-galactosidase. Results: AMCAP™ proved high mRNA production yield comparable to both traditional control processes. High-resolution mass spectrometry confirmed that AMCAP™ achieves superior 5'CAP efficiency. Crucially, AMCAP™ also drastically reduced dsRNA contamination levels, lowering the risk of unwanted immunogenicity. The technology showed remarkable process flexibility, maintaining robust performance across multiple commercial reagent mixes and different template starts. Furthermore, functional assays confirmed high protein expression and enzymatic activity across all tested targets. Conclusion: AMCAP™ offers a smarter, faster, and effortless alternative for mRNA manufacturing. By integrating transcription and capping into a single enzymatic step, it halves downstream purification operations and lowers production costs per batch. AMCAP™ represents a major advancement in mRNA manufacturing by consolidating essential steps without compromising product integrity. Optimized for a seamless transition from R&D to GMP environments, this technology is uniquely positioned to accelerate the journey from laboratory to clinical implementation.