

## LEUCINE AND ISOLEUCINE DERIVATIVES AS BIOAVAILABLE ALTERNATIVES FOR CANONICAL AMINO ACIDS IN CELL CULTURE MEDIA TO ENABLE NEXT GENERATION BIOPROCESSES

Tim Hofmann, Merck, Upstream R&D  
Tim.Hofmann@merckgroup.com  
Corinna Schmidt, Merck, Upstream R&D  
Dmitry Zabezhinsky, Merck, Upstream R&D  
Aline Zimmer, Merck, Upstream R&D

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The increasing demand of protein based biotherapeutics such as monoclonal antibodies, complex recombinant bispecific antibodies, antibody-based fusion proteins and antibody fragments requires innovative solutions and the constant improvement and optimization of bioprocesses. Large quantities of biotherapeutics need to be manufactured in a cost- and time efficient manner in order to obtain large doses of drugs for clinical trials and commercialization. Concentrated cell culture medium formulations are one way to increase volumetric productivity in the generation of biotherapeutics by adding less feed volume or using existing bioreactor capacity more efficiently. However, state-of-the art feeds cannot be further concentrated due to limited solubility of amino acids such as tyrosine, cystine, leucine and isoleucine, in particular at physiological pH. A modification of canonical Leucine and Isoleucine to Keto-(Iso)Leucine and N-lactoyl-(Iso)Leucine sodium salts allow to increase the overall solubility of cell culture media and/or feed formulations. These modified amino acids were shown to be bioavailable in several chinese hamster ovary (CHO) cell lines and were designed to be a suitable replacement for their canonical counterparts. This unlocks the possibility to generate highly concentrated cell culture feed formulations which will in return increase the protein output and volumetric productivity per batch, fed-batch or perfusion through higher bioreactor starting volumes, decreased feed volumes and increased cell masses. Cell specific productivity and critical quality attributes like post translational modifications, aggregation and fragmentation were not negatively impacted, while maintaining or improving cell culture performance. Next generation cell culture formulations including bioavailable highly soluble modified amino acid derivatives like Keto-(Iso)Leucine and N-lactoyl-(Iso)Leucine, will satisfy the pharma industrial demand and ultimately leading to better bioprocesses.