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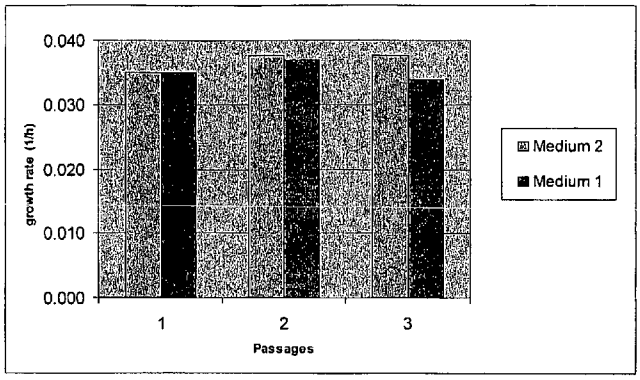
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(54) **Title:** PRODUCTION OF TNFR-Ig FUSION PROTEIN

Comparison of Medium 1 and Medium 2 in shake flasks using anti-GDF-8 cells.



(57) **Abstract:** An improved system for large scale production of a dimeric fusion protein consisting of the extracellular ligang-binding portion of the human (p75) tumor necrosis factor receptor (TNFR) linked to the Fc portion of IgGI in cell culture, particularly in media characterized by one or more of: i) a cumulative amino acid concentration greater than about 70 mM; ii) a molar cumulative glutamine to cumulative asparagine ratio of less than about 2; iii) a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2; iv) a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1; or v) a combined cumulative glutamine and cumulative asparagine concentration between about 16 and 36 mM, is provided. The use of such a system allows high levels of protein production and lessens accumulation of certain undesirable factors such as ammonium and/or lactate. Additionally, culture methods including a temperature shift, typically including a decrease in temperature when the culture has reached about 20-80% of it maximal cell density, are provided. Alternatively or additionally, the present invention provides methods such that, after reaching a peak, lactate and/or ammonium levels in the culture decrease over time.

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