GENERATION AND CHARACTERIZATION OF A PROCESS SPECIFIC HCP REAGENT USING STABLY TRANSFECTED NULL CELL POOLS

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During early clinical evaluation of a therapeutic protein expressed in mammalian cells, host cell protein (HCP) content may be quantified using commercially available, generic reagents such as anti-CHO-HCP ELISA kits. However, during late-stage clinical development and for commercialization regulatory agencies require the development and characterization of process-specific HCP reagents which should provide better coverage of the HCP population in a given process when compared to commercially available reagents. The derivation of a process-specific HCP reagent is not trivial; multiple different approaches have been suggested.

Here, we report the generation of stably transfected cell pools from the same host cell pool as the producer cell line. We employed a plasmid vector harboring the same principal features as the original transfection vector, except the expression cassette for the therapeutic protein, and used it to raise six distinct null cell pools. We characterized the pools in batch exhaustion experiments using the same cell culture media and other raw materials as utilized in the production process. We identified differences in doubling times, viability profiles, and metabolic profiles including specific glucose consumption rates. Furthermore, we tested all six pools under slightly varied process conditions in an AMBR15 miniature bioreactor system to screen for process conditions that would generate cumulative cell mass and viability profiles similar to those of the producer cell line in the 14-day center point fed-batch process. From these screening experiments, we identified several key factors which influence integrated viable cell density (IVCD) and harvest viability, including seeding density, temperature, and allowable pH range. Interestingly, the six null cell pools segregated into two distinct groups. We employed a 2 L single-use bioreactor system and used one representative pool of each group and ran each under three slightly varied process conditions. These six 2 L bioreactors were harvested once the viability of the null cell pool cultures was in range of the harvest viability of the 14-day production process. All null cell pools reached harvest viability sooner than the 14-day mark. Cultures were removed from the bioreactor, and cell pellets collected by centrifugation and discarded. The supernatant was dead-end filtered through a 0.2 µm sterilizing filter and stored at -80°C for further analysis.

Quantitative HCP analysis revealed that sufficient material was generated to proceed to HCP reagent production from any one bioreactor, or pool of bioreactors. The material was screened by 2-dimensional SDS-PAGE analysis and compared to the HCP profile in the unprocessed harvest bulk of the production cell line at the end of the center point process.