

# A Rapid and Effective Screening Process of Animal Component Free Hydrolysates to Increase Cell Performance

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## ABSTRACT

Different cell lines exhibit a large degree of variability in their nutritional requirements. As a result, each transfected cell line has its own unique set of nutritional requirements for maximizing growth and recombinant protein production. With current regulatory standards, a chemically defined medium is the ideal choice. However, product development, production goals, and production schedules do not always allow for complete optimization of a chemically defined medium. Both time and production related issues can be successfully addressed by using an adequate chemically defined base medium supplemented with animal free hydrolysates.

Through the implementation of a unique process utilizing mixture designs, we developed two animal free hydrolysate containing media, which increased the production of recombinant mAb from two different CHO cell lines. These data demonstrate our ability to increase production levels as much as 2-fold in less than a 3-month period. The benefit of blends of hydrolysates over individual hydrolysates is also demonstrated.

## MATERIALS AND METHODS

**CELL LINES:** CHO cell line 1 and CHO cell line 2, both producing human IgG.

**CULTURE MEDIA AND SUPPLEMENTS:** CHO medium with supplements, Medium B (Proprietary Chemically Defined Basal Medium) BD Peptones: Select Soytone, TC Yeastolate UF, Yeast Extract UF, Phytone™ Peptone UF, Difco Springer DS100 Soy peptone, Phytone™ Peptone, Proteose Peptone 3

**EQUIPMENTS AND REAGENTS:** Radial Immuno diffusion plates (RID, The Binding Site), Alamar Blue™ (BioSource), Trypan blue (Gibco), Falcon microtiter plates (Becton Dickinson), 125 and 500 mL shaker flasks (Corning).

**CULTURE CONDITIONS:** Initial cell density:  $6.25 \times 10^4$  cells/mL per well of 96-well microtiter plates and  $2 \times 10^5$  cells/mL in 20 mL medium in 125 mL shaker flasks. Cells grown in a humidified CO<sub>2</sub> incubator set at 5% CO<sub>2</sub> air saturation.

**ANALYTICAL METHODS:** Cell proliferation: Alamar Blue™ assay, Cell Count: Cedex Cell counter (Innovatis GmbH) Metabolite chemistry: NOVA Bio-profile 400 (NOVA Biomedical) MAb production: Human IgG ELISA, RID and Protein A HPLC (Waters 2695).

## RESULTS

### Development of Animal Component free Medium for CHO Line 1

Based on our data from earlier media screening experiments, Medium B was selected for use in the peptone screening studies. Six animal free peptones were screened in 96-well microtiter plates in Medium B using a unique proprietary mixture design generated by the DOE platform of the statistical software. Figure 1 shows day 5 proliferation data from CHO Line 1 with the six peptones that were blended together in 91 unique blends according to the design generated by the statistical DOE. Figure 2 shows the IgG production as measured by ELISA. Medium B without peptones was included as a control. This data was analyzed using statistical software to pick the top three performing peptones at their best concentrations.

Three peptones (Select Soytone, TC Yeastolate UF and Yeast Extract UF) were found to have the greatest enhancement of growth and antibody production, either independently or as a mixture. These three peptones were further screened using a mixture design to identify the single best performing peptone or a mixture of peptones and the optimal blending ratio. This evaluation was performed in shaker flasks in a ten day batch culture and included Medium B without peptones as a control. Trypan blue viable cell concentrations and antibody production from day 10 supernatant are shown in Figures 3 and 4.

### Development of Animal Component free Medium for CHO Line 2

Using the same peptone screening process described above, we developed a second animal free medium for CHO Line 2. The initial platform and the screening design used for the screening the six animal free peptones were same as that used for CHO Line 1. Using 96-well microtiter plates, six animal free peptones were screened in CHO medium. Figure 5 shows the proliferation data of CHO Line 2 in response to various blends of the six peptones. Figure 6 shows the day 7 IgG production data as determined by ELISA. CHO medium without peptones was included as a control. Following data analysis by the statistical software, three peptones (DS 100 Soy peptone, TC Yeastolate UF and Yeast Extract UF) were found to have the greatest enhancement of growth and antibody production, either independently or as a mixture. These three peptones were further screened using a mixture design to identify the single best performing peptone or a mixture of peptones and the optimal blending ratio. This evaluation was performed in shaker flasks in an eleven day batch culture and included CHO medium without peptones as the control. Viable cell concentrations and antibody production from day 11 supernatant samples are shown in Figures 7 and 8.

## DISCUSSION

Once the peptone-supplemented media mixing assays were completed, the peptone mixing data was analyzed using a Design-of-Experiment (DOE) software package. Based on the data inputs, mathematical models were used to predict the outcome of a number of combinations of the three peptones with the basal media resulting in various blends. In addition, desirability of each mixture was determined. The final outcome was one of several best-fit media designed specifically to meet the nutritional requirements of the cell line under investigation.

Analysis of CHO Line 1 shaker data showed that Medium B supplemented either with a single peptone or a blend of two to three peptones produced a 2-fold increase in antibody production

as compared to the control. Based on the statistical analysis of the data and DOE predictions, Medium B with a blend of Select Soytone and Yeast Extract UF resulted in a 2.4 fold increase in antibody production as compared to the control-Medium B without any peptones. Similarly, analysis of CHO Line 2 shaker data showed that CHO medium supplemented either with a single peptone or a blend of peptones produced in a 1.5-fold increase in antibody production as compared to the control. Again statistical analysis of the data and based on the DOE predictions, CHO medium with a blend of TC Yeastolate UF and Yeast Extract UF resulted in a 2.7 fold increase in antibody production as compared to the control CHO medium without any peptones.

Figure 1. CHO Line 1 Peptone Screen — Day 5 Alamar Blue Proliferation Data

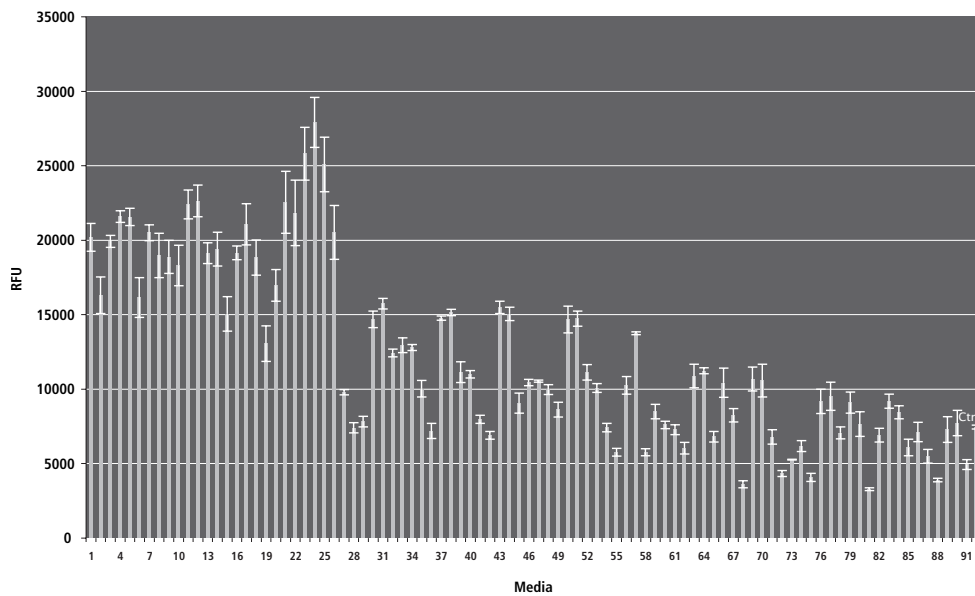


Figure 2. CHO Line 1 Peptone Screen — Day 7 ELISA Hu IgG Production Data

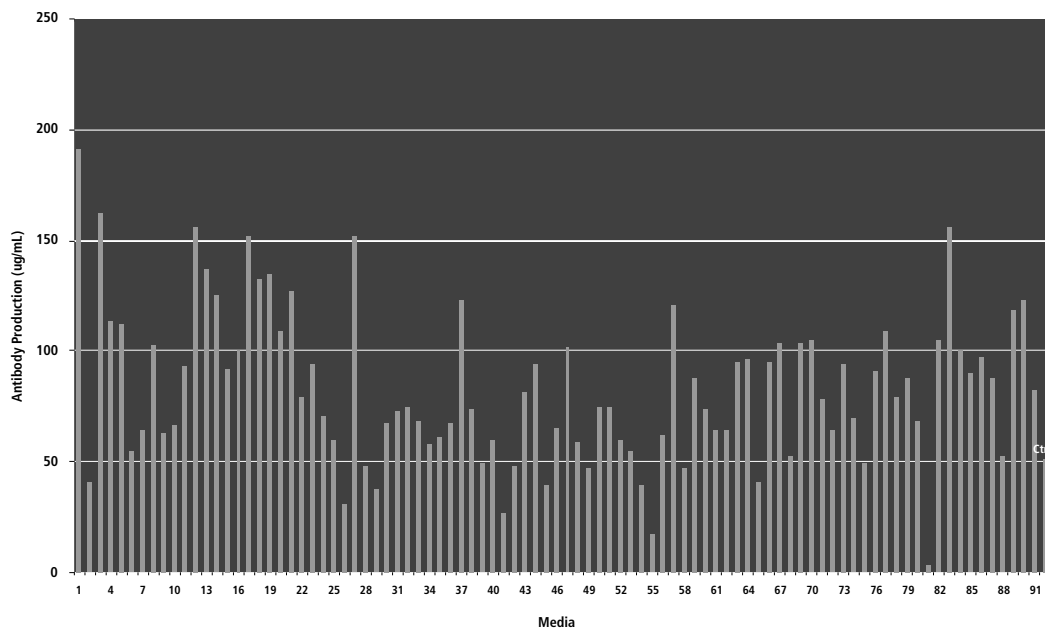


Figure 3. CHO Line 1 Shaker Study — Trypan Blue Viability Cell Concentration

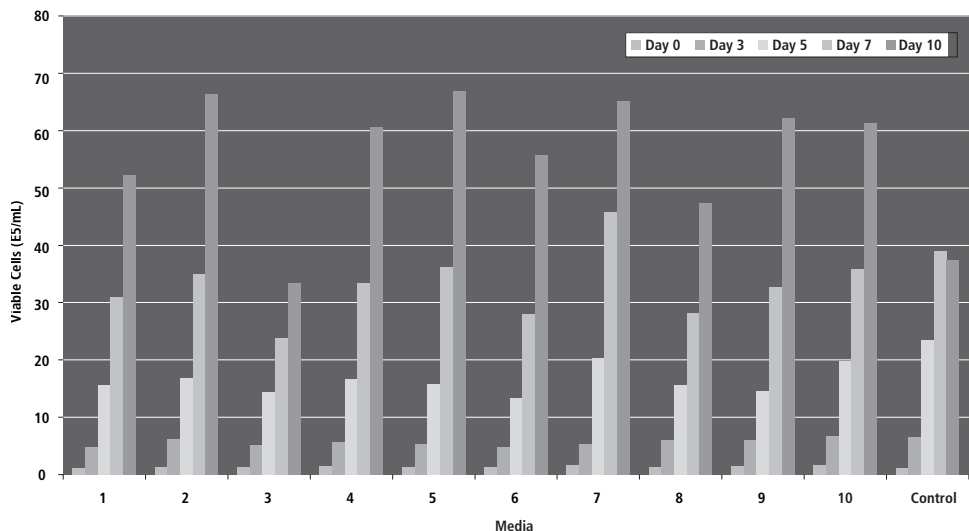


Figure 4. CHO Line 1 Shaker Study — Day 10 RID Human IgG Production Data

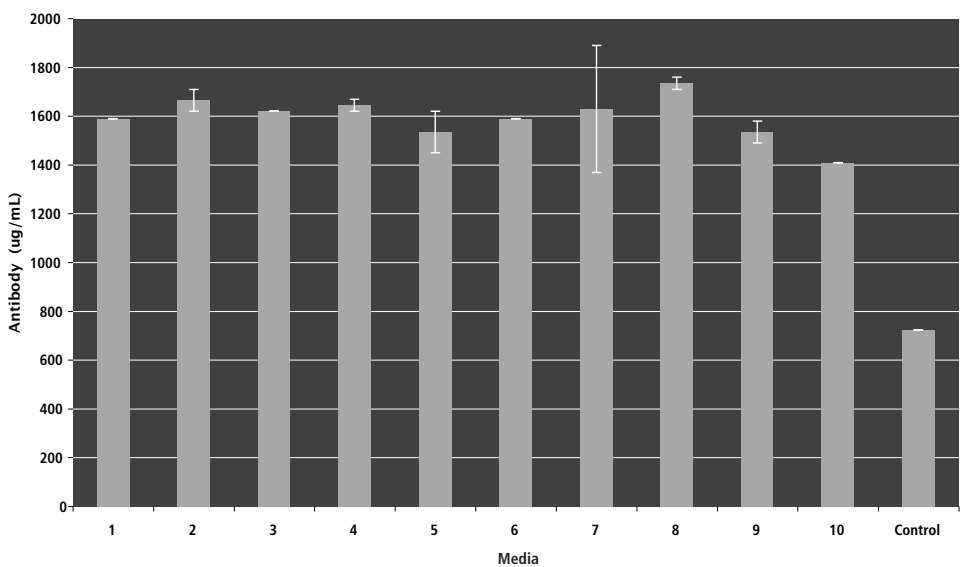


Figure 5. CHO Line 2 Peptone Screen — Day 7 Alamar Blue Proliferation Data

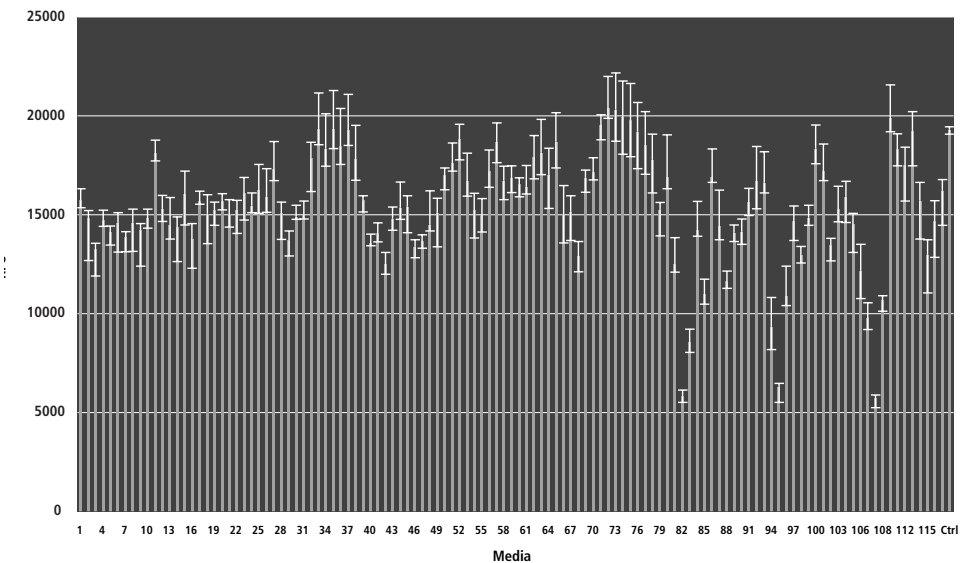


Figure 6. CHO Line 2 Peptone Screen — Day 7 ELISA Human IgG Production

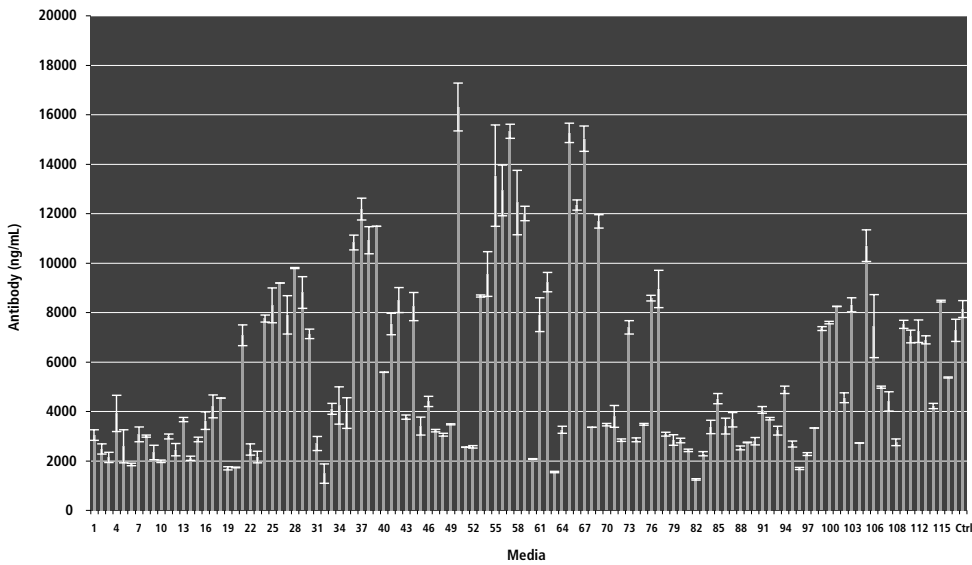


Figure 7. CHO Line 2 Shaker Data—Trypan Blue Viable Cell Concentration

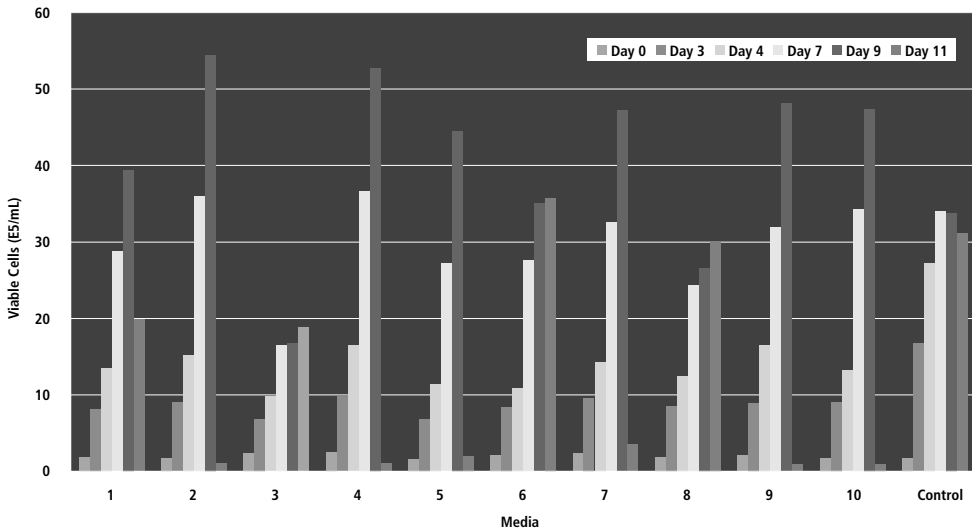
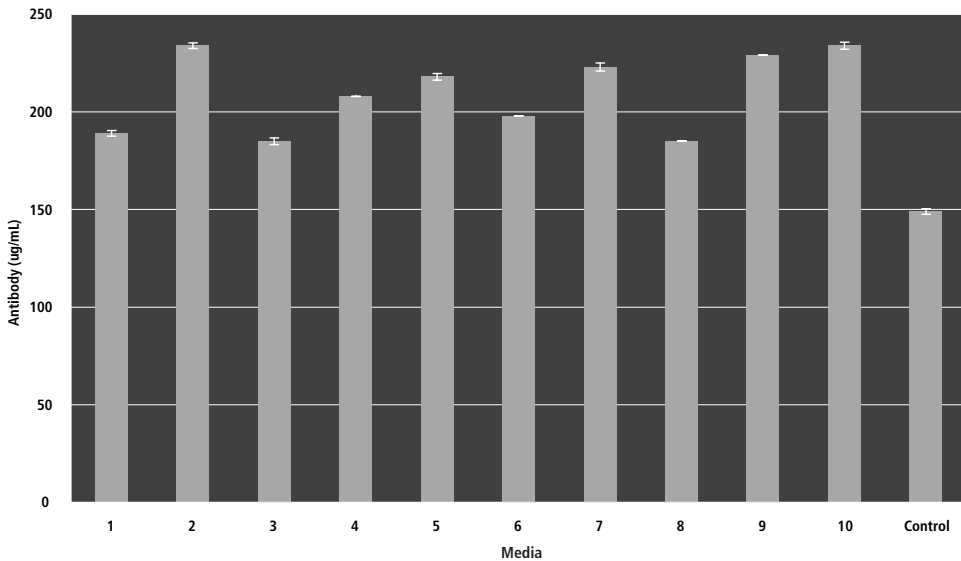


Figure 8. CHO Line 2 Shaker Study—Day 11 RID Human IgG Production



### CONCLUSIONS

As more and more recombinant CHO clones have been developed, it has become increasingly important to streamline the medium optimization process. The convenient format of the screening protocol described here allows for a rapid screening of multiple animal free peptones supplemented in a basal medium of choice. In addition, the powerful mixture designs generated by the DOE platform of the statistical software provide an invaluable tool for finding ideal formulations of the peptones with the basal medium, boosting cell growth and productivity. Looking at the time constraints product development and production has, our peptone screening process can help increase production levels by as much as two fold in less than a 3-month period. In conclusion, this peptone screening protocol can be conveniently adapted to any cell line, apart from CHO cell lines, to increase their cell performance significantly.