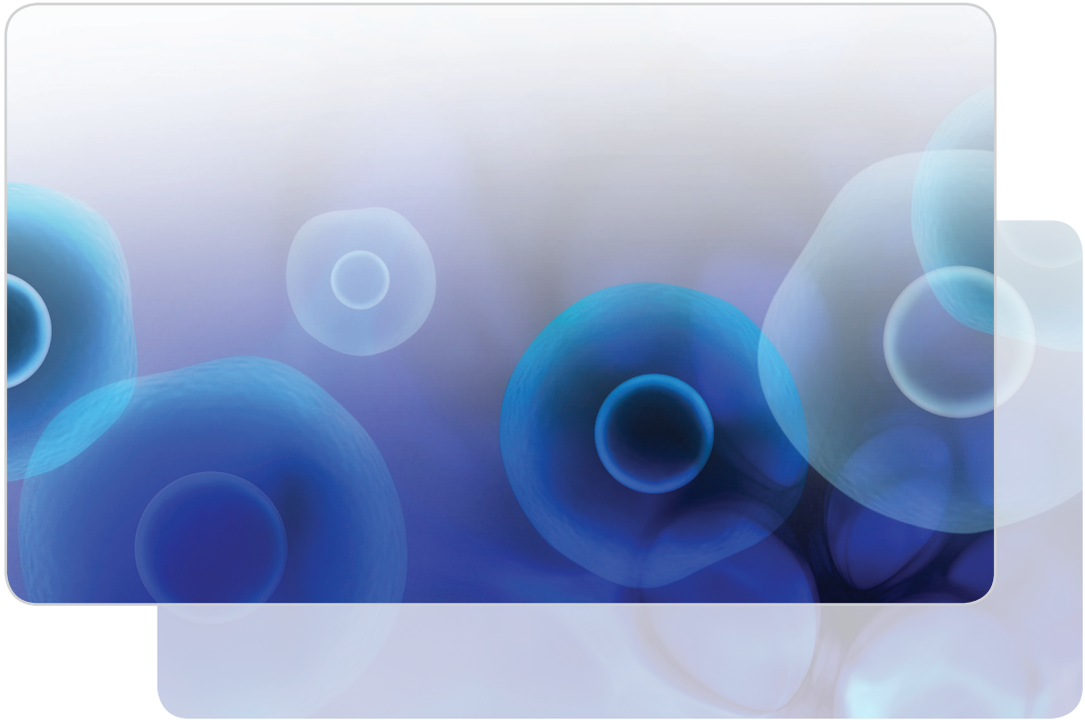
BD Recharge™
without Glucose and L-Glutamine



User Manual

BD
Helping all people
live healthy lives

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1 Introduction

Cell-based productions of active pharmaceutical ingredients generally require supplementation of cell culture media to promote cell growth and boost protein production. In the ideal world, cell culture processes would have both a chemically defined base medium and a chemically defined supplement. Modern animal free cell culture technologies utilize chemically defined media and may also use animal free (yeast and/or soy–based) or animal origin peptones as supplements in the bioproduction process. While very useful as supplements, peptones, by definition, are undefined biological products and thus, may contribute to lot-to-lot bioprocess variation.

To develop a chemically defined supplement, we employed proprietary analytical technologies to characterize a yeast extract peptone and identify active components that drive protein production. Based on this work, BD Biosciences – Advanced Bioprocessing has designed and formulated a fully chemically defined, animal free cell culture supplement, BD Recharge™ without Glucose and L-Glutamine (BD Recharge), which can be used as an alternative to peptones for CHO cell lines.

2 Product Information

BD Recharge is not a traditional glucose-containing feed; it is a supplement that should be added on Day 1 of a production culture to boost cell performance from the outset.

BD Recharge is a chemically defined supplement which can be used as an alternative to a yeast extract peptone and is designed for batch or fed-batch processes with CHO cell lines. The product is currently offered in the following configurations:

Table 1. Product Information

Part Number	Package Size	Shipping Temperature	Recommended Storage
670002	100 g	Ambient	2-8˚C
670003	1 kg	Ambient	2-8˚C
670004	5 kg	Ambient	2-8˚C

BD Recharge is manufactured at the BD Biosciences – Advanced Bioprocessing animal free and antibiotic free (AF^2), state of the art facility in Miami, Florida. All the components are chemically defined with a known molecular formula and structure and meet Becton Dickinson's (BD) Animal Origin Free Specification.

BD Recharge has demonstrated comparable cell growth characteristics and protein production compared to a yeast extract peptone in CHO cell lines expressing monoclonal antibodies and recombinant proteins. (For more information, go to bdbiosciences.com/go/recharge to download a white paper.) BD Recharge offers analytical lot-to-lot consistency beyond what is possible for a peptone and stability that is attractive for pharmaceutical and bioproduction applications.

When reconstituted as described below, BD Recharge will have an osmolality of 445 ± 30 mOsmo/Kg. When supplemented to basal medium, the osmolality contribution of BD Recharge to the final mixture (BD Recharge and base medium) on a gram for gram basis is lower than the contribution from a Yeast Extract Peptone (Table 2). And since BD Recharge is often used at a lower (gram for gram) concentration than Yeast Extract Peptone, there is even less of an impact on the final complete medium.

Table 2. Osmolality Contribution by BD Recharge and Yeast Extract

Concentration in g/L	Osmolality Contribution of BD Recharge	Osmolality Contribution of Yeast Extract Peptone
4.342	13.665	21
8.684	27.33	48

3 Reconstitution Procedure

Final Volume: 1L of BD Recharge Concentrated Solution

3.1

Materials

- BD Recharge: 43.42 g
- Clean 1000 mL (1 L) beaker
- Measuring cylinder or volumetric flask
- Water for Injection (WFI) or equivalent
- Polyethersulfone (PES) 0.2 µm filter unit or equivalent
- Stir plate, stir bar, pH meter, osmometer

3.2

Procedure

a. Fill beaker with 80% of the final volume of water (800 mL) and begin stirring.

b. Weigh and add 43.42 g of BD Recharge powder to the beaker.

c. Mix until completely dissolved.

d. Bring the volume to 1000 mL (1L) with water and mix further or a minimum of 10 minutes.

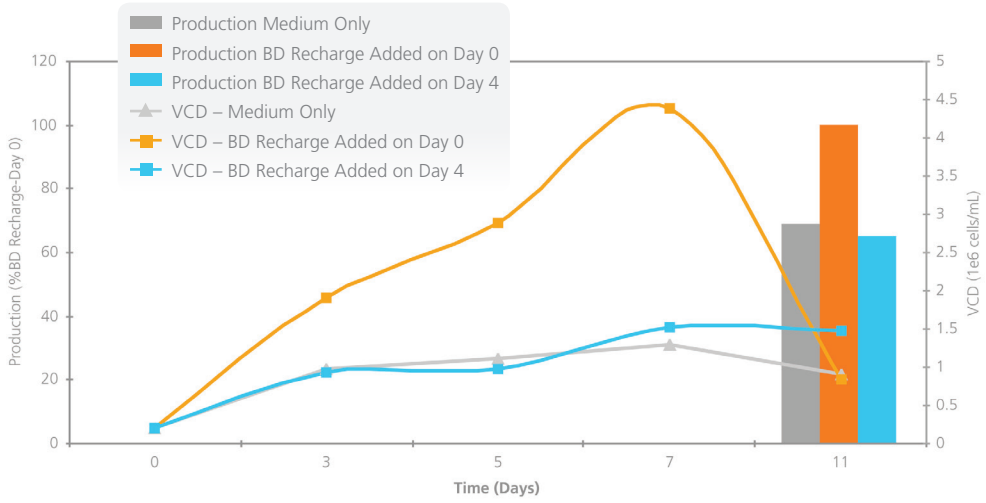
e. Record pH (6.5 – 7.0) and Osmolality (445±30 mOsm/Kg).

f. Filter the mixture immediately through a 0.2 µm Polyethersulfone (PES) filter unit (or equivalent) and store at 2-8°C in the same filter unit or transfer to a sterile bottle.

4 Application Protocol

BD Recharge is designed for CHO cell lines responsive to yeast extract peptones and should be added in the base medium at the start of culturing for optimal results (Figure 1). It is recommended that an evaluation be conducted to determine optimal culture parameters and conditions: volume, seeding density, sampling days, feed days, etc. This supplement does not contain glucose and glutamine, so it is recommended that glucose and glutamine be monitored and supplemented, as appropriate. A titration should be performed to determine optimal concentration of BD Recharge for each cell line (See section 4.2). For quick screening, BD Recharge can be used at a yeast peptone equivalent concentration. Refer to Table 3 for BD Recharge equivalency to yeast extract peptone.

Figure 1. Growth and Production Profile for Adding BD Recharge in Cell Culture at Day 0 Compared to 4



4 Application Protocol

4.1

Materials for a Typical Cell Culture Testing Protocol

a. Base Media, supplemented with all required components, EXCEPT hydrolysates.

b. BD Recharge stock solution (43.42 g/L) from section 3.2 above.

c. 10% Hydrolysate Stock (positive control), sterile filtered (100 g/L).

d. Additional cell culture supplies and equipment as needed, such as instrumentation for analysis of cell number, viability, glucose, glutamine and protein production, sterile Erlenmeyer shake flasks, disposable sterile pipettes, serological pipettes and micropipettors.

4.2

BD Recharge Titration: Batch Culture

Small scale (50 mL) batch culture are set-up by preparing experimental shaker flasks, in duplicate, and adding an appropriate volume of the BD Recharge stock solution to result in the final concentrations shown in Table 3 below. Table 3 also shows the amount of BD Recharge equivalent to yeast extract peptone. The peptone control shaker flasks can be set up using the optimal concentration of peptone established for the current culture process. The titration data from BD Recharge can then be compared with the positive control to establish the optimal BD Recharge concentration.

Table 3. Amounts of BD Recharge Concentrate Required for Testing

Shaker ID	BD Recharge (g/L)	Peptone Equivalent Concentration (g/L)	Volume of Base Medium (mL)	Volume of Concentrated BD Recharge (mL)
1	0.58	1.00	50.00	0.67
2	1.74	3.00	48.00	2.00
3	3.47	6.00	46.00	4.00
4	5.21	9.00	44.00	6.00
5	6.25	11.00	42.80	7.20
6 Negative Control	0	0	50.00	0
7 Positive Control	0	Optimal Peptone Concentration	Calculate	0

Note – This table provides guidance for initial studies; more titration points or replicates can be added

4 Application Protocol

4.2 Sample Titration Protocol

a. Prepare seeding culture according to standard protocol (e.g. 3 x 10^5 cells/mL).

b. If cells are being cultured in medium containing hydrolysate, wash cells once in PBS and pellet by centrifugation.

c. Prepare seeding cell suspension by resuspending pellet into base media (e.g. 3x10^7 cell/mL).

d. Inoculate shaker flasks with usual seeding density (e.g. 3x10^5 cell/mL).

e. On Days 0, 3, 5, 7, 10, and last day of cell culture, perform cell counts to determine viable cell density and percent viability.

f. Perform appropriate readings and adjust glucose and glutamine levels as appropriate throughout the experiment.

g. Determine protein titer on various days of culture including last day to determine total protein production.

4.3 Fed-batch Culture

The recommended supplement range is 0.5 g/L – 5.21 g/L or specific yeast extract peptone equivalent concentration for the test cell line (Table 3). Feeding strategy should include adding BD Recharge at Day 0 and again at one or several times into late growth phase. As each cell line may require a different optimal concentration, it is recommended to first perform a titration study using a batch culture to get information on productivity and viability using BD Recharge. If a feeding strategy is known for a peptone, a similar scheme can be used for BD Recharge with addition of BD Recharge on Day 0 if it is not a part of the current peptone feed protocol. However, it is recommended to further optimize BD Recharge feed for the specific cell line. Below (Table 4), is a suggested initial feeding scheme based on representative results of titration studies and assuming that batch culture titrations study of BD Recharge for a CHO line fall into the following experimental parameters:

1. Optimal protein production levels attained at 1.74-3.47 g/L concentration of BD Recharge
2. BD Recharge concentrations greater than 5.21 g/L showed toxic/detrimental effect on cell growth and viability
3. The total days of culture were greater than or equal to 10

4 Application Protocol

4.3

Table 4. Suggested Feeding Guidelines

Scheme #	Day 0	Day 3	Day 5	Day 7	Day 9
1	1.74 g/L	Feed 0.87 g/L per day on three additional alternate days. e.g. Day 3, 5, and 7 Total BD Recharge ≤ 3.74 g/L			
2	1.74 g/L	Feed 1.74 g/L per day on two additional alternate days. e.g. Day 5 and 7 Total BD Recharge ≤ 5.2 g/L			
3	3.47 g/L	Feed 1.74 g/L on one additional day. e.g. Day 5 Total BD Recharge ≤ 5.2 g/L			
4	1.74 g/L	Feed 3.47 g/L on one additional day. e.g. Day 5 Total BD Recharge ≤ 5.2 g/L			

Note – As individual cell lines will respond differently to BD Recharge, the table above provides guidance for initial studies but optimal results may require cell-line specific feed strategies.

Glucose, glutamine, lactate, and ammonia levels should be monitored separately throughout the culture. Glucose should be fed as required for the specific cell line; typically BD Recharge gives optimal performance in the presence of at least 3 g/L glucose. Glucose feed optimization is required to achieve optimal performance.

4.4

Scale Up to Bioreactor

Select two or three optimal concentrations of BD Recharge from the shaker flask batch culture study for bioreactor batch study. Monitor and control glucose and glutamine levels as defined in your current process. Monitor process variables as well as metabolites including lactate and ammonia to assess cell culture performance. For fed batch processes in bioreactors, it is recommended to test a minimum of two feeding schemes that showed promising results in the shaker flask. Further optimization of the feeding scheme should be done based on metabolite levels and the bioreactor system.

5 Compatibility Studies

5.1 Overview

For troubleshooting any compatibility issues experienced while using a supplement with a basal medium in cell culture processes, it is important to determine the effect of the supplement on basal medium characteristics. A supplement would be considered compatible with the basal medium if the following characteristics are preserved (Table 5):

Table 5. Required Compatibility Characteristics of BD Recharge

Characteristic	Requirement	Notes
Chemical Interaction	No color change due to supplement addition to cell culture medium	Color change due to addition of supplement may suggest presence of chemical reactions
Osmolality	Should not be significant to affect osmolality of basal cell culture medium	Use base medium osmolality and the product information above (Table 2) to determine suitability
pH	Should not be significant to affect the pH of cell culture medium	pH specification of the supplement is 6.5 to 7.0
Precipitation	No precipitate formation due to supplement addition to cell culture medium	Observed visually after addition of the supplement to the basal medium. Precipitation would indicate solubility issues or chemical reaction

5 Compatibility Studies

5.2

Compatibility with Base Medium Testing Procedure

The following procedure should be followed to test the compatibility of BD Recharge with a basal medium:

a. Reconstitute BD Recharge as specified in section 3.2.

b. Titrate the basal medium with the reconstituted BD Recharge according to Table 6 below.

Table 6. Compatibility Testing Guidelines

Basal Medium (mL)	mL of Reconstituted BD Recharge	Corresponding g/L of BD Recharge
1000	0	0
980	20	0.8684
960	40	1.7368
940	60	2.6052
920	80	3.4736
900	100	4.342
800	200	8.684
600	400	17.368

c. Record the osmolality and pH of each mixture and determine the level of BD Recharge that would give the desired characteristics.

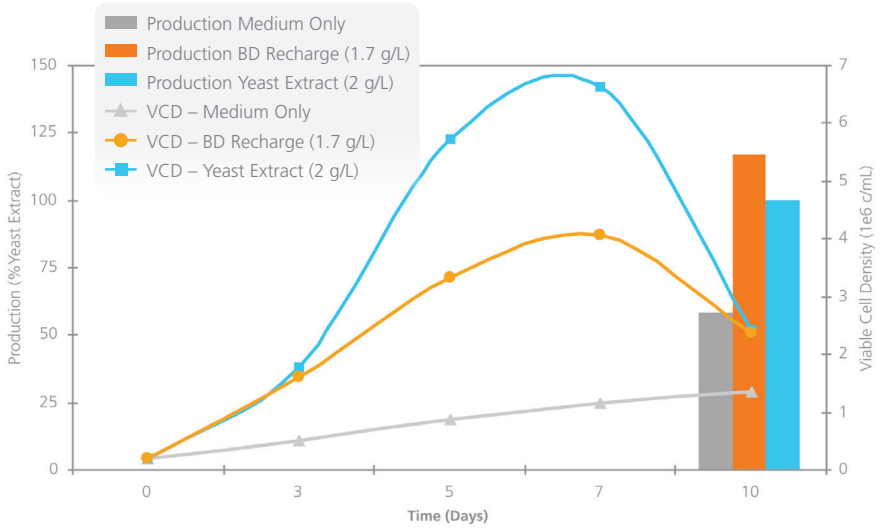
d. Store the mixture protected from light at temperatures above the recommendation storage condition (e.g. 35.5˚C to 42˚C) and observe for precipitation or color change over 24-48 hrs.

e. Use the information obtained from steps c & d above to establish the amount of BD Recharge that is compatible with the basal medium, as per Table 5.

6 Representative Data

Performance of BD Recharge was compared to yeast extract peptone in a shaker flask for a monoclonal antibody producing CHO-K1 line (Figure 2). BD Recharge increased growth compared to the Production Medium only control and increased specific productivity as compared to yeast extract peptone. The viable cell density (VCD) for BD Recharge was lower than with yeast extract peptone, but the protein production obtained by using BD Recharge was higher than with yeast extract peptone. Note: BD Recharge and yeast extract peptone concentrations were independently optimized for this cell line.

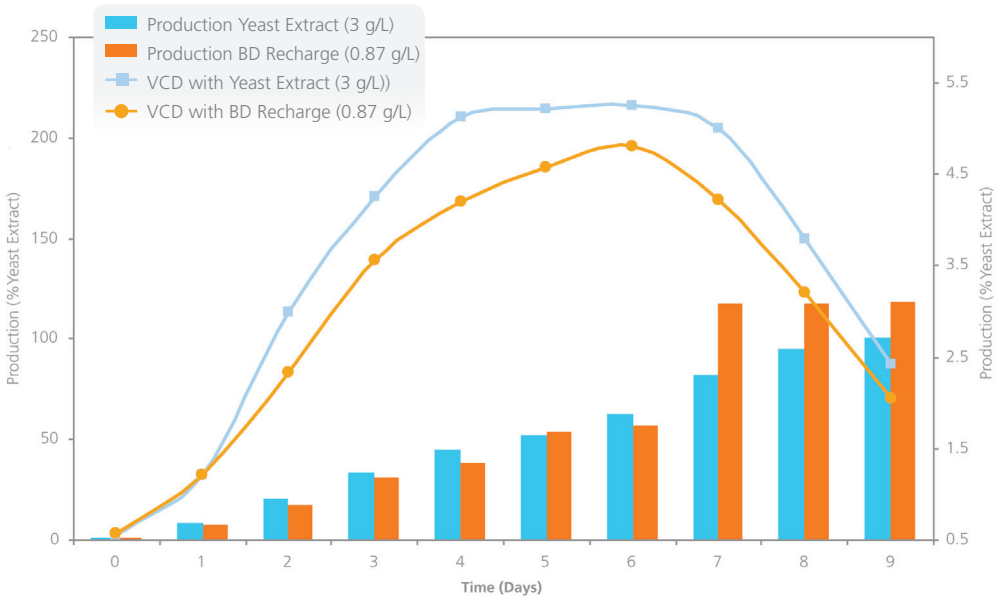
Figure 2. Viable Cell Density and Production Profiles of BD Recharge vs. Yeast Extract in Shaker Flask



6 Representative Data

Viable cell density and production for this cell line was also compared in bioreactors with BD Recharge at 0.87 g/L and yeast extract peptone at 3 g/L (Figure 3). Glucose was monitored and controlled through out the culture. Similar to shaker flask conditions, specific productivity was also positively impacted; the cell growth was slightly lower with BD Recharge than yeast extract peptone while production levels were comparable in the exponential and stationary growth phases. Additionally, production levels in the late phase of the culture are higher for BD Recharge.

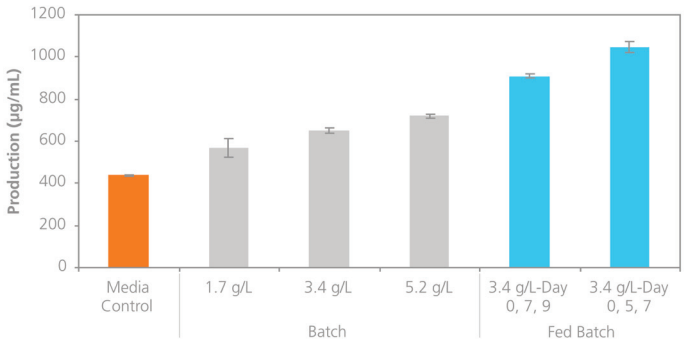
Figure 3. Viable Cell Density and Production Profiles of BD Recharge vs. Yeast Extract in Bioreactor



6 Representative Data

Figure 4, shows fed batch results for another mAb-producing CHO cell line cultured in shaker flasks. Improvement in protein production over batch conditions was seen when the BD Recharge was fed three times during the run, with the best performance shown by feeding on Days 0, 5, and 7. The level and mode of supplementation (batch *vs.* fed-batch) are indicated along the x-axis. Error bars indicate ±1 standard deviation for duplicate flasks.

Figure 4. Protein Production Using a Batch vs. Fed-batch Approach with BD Recharge



7 Troubleshooting

Table 7. Trouble Shooting Guidelines

Problem	Cause	Solution
BD Recharge either not dissolving or taking excessively long time to completely dissolve	Low vortex due to slow mix speed or wrong reconstitution vessel	• Use beaker to reconstitute small amounts of BD Recharge • Increase mixing speed to maximize vortex
	Temperature of water or room is unusually low	• Reconstitute with room temperature (~ 25°C) water in an environment controlled at ~ 25°C
Medium is cloudy or a precipitate is observed following addition of BD Recharge	Possible reaction between components resulting in undissolved products. Concentration of some components above solubility limits	• Perform compatibility test to identify correct concentration of BD Recharge to use • Contact BD Technical Services for trouble shooting unwanted chemical reactions
	Possible contamination	• Identify contaminant and root cause of contamination • Retest using aseptic procedures
Color of medium containing BD Recharge changes during storage	Possible reaction between components or degradation of the components resulting to fluorescent by-products	• Contact BD Technical Services for trouble shooting unwanted chemical reactions
High osmolality and pH after addition to medium	High concentration of BD Recharge used	• Perform compatibility test to identify the optimum concentration of BD Recharge to use for your application
Low cell growth and low cell viability	High levels of BD Recharge	• Perform BD Recharge titration study to determine concentration range for BD Recharge's performance • Re-check calculations for amount of BD Recharge added
	High osmolality due to use of other additives like amino acid feeds, hydrolysates feeds etc.	• Perform compatibility test to identify the optimum concentration of BD Recharge to use for your application • Reduce other supplementation levels
	Over supplementation if using other feed additives (amino acids, hydrolysates etc) together with BD Recharge	• Try alternate feeding plan with reduced amount of other feeds (amino acids, hydrolysates etc.)
	BD Recharge not supplemented on Day 0 of culture	• Add BD Recharge on Day 0 of culture for optimal performance

7 Troubleshooting

Table 7. Trouble Shooting Guidelines

Problem	Cause	Solution
Low protein production	Low cell growth and low cell viability	• See "Low cell growth and low cell viability" section
	Not sampling on the day of peak protein production	• Sample multiple time points until cell viability decreases below 50% in order to determine time of peak protein production
	BD Recharge not supplemented on Day 0 of culture	• Add BD Recharge on Day 0 of culture for optimal performance
Rapid cell growth and low protein production	Energy shifted towards cell growth	• Try alternate feeding pattern • Shift temperature to reduce cell growth • Use cells from log phase of culture (when cells are in their growth phase) when seeding the cells • Use high seeding density

8 Summary and Recommendations

The data presented in this protocol have demonstrated that BD Recharge can easily be reconstituted without pH adjustments to generate a concentrated chemically defined supplement solution. This concentrated solution has minimal osmolality and pH impact on cell culture basal medium.

BD Recharge is animal free, protein free, and chemically defined, and can directly replace yeast extract peptones in cell culture processes. It has demonstrated comparable cell growth characteristics and protein production in CHO cell lines expressing monoclonal antibodies and recombinant proteins. Supplementation with BD Recharge is scalable, and has been demonstrated to perform in spin tubes, shake flasks and bioreactors.

BD Recharge can be used as a replacement for a yeast extract peptone in cell culture processes designed for yeast extract peptone responsive CHO cell lines. Although the development of BD Recharge focused on yeast extract peptone responsive CHO cell lines expressing monoclonal antibodies and recombinant proteins, its application can certainly be investigated on other host cell lines, as well as any cell lines that are responsive to non-yeast based supplementation.

9 References

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