carrier). The polypeptide formulations in one embodiment have an acceptable color and maintain a protein-based drug product concentration of at least 100 mg/mL or 150 mg/mL.

[0008] The cell culture media detailed herein generally comprise one or more of the following components in an amount to effect a protein product quality attribute such as color: (a) cystine or cysteine; (b) vitamin B2, (c) vitamin B6 (pyridoxine and/or pyridoxal, which may be provided as the HCl salt), (d) vitamin B9, (e) vitamin B12, (f) an iron source such as iron nitrate, ferric citrate or ferrous sulfate and (g) hydrocortisone. In one variation, a cell culture medium comprises 2 or 3 or 4 or 5 or 6 or each of components (a), (b), (c), (d), (e), (f) and (g). It is understood that a cell culture medium provided herein may contain any combination of components (a), (b), (c), (d), (e), (f) and (g) the same as if each and every combination were specifically and individually listed. In one embodiment, the cell culture media is a CDM. In another embodiment, the cell culture media is chemically undefined.

[0008a] ~~In one aspect, there is provided~~Disclosed is a cell culture medium comprising:

from about 200 mg/L to about 1200 mg/L cystine;

from about 0.05 mg/L to about 1.0 mg/L vitamin B2;

from about 0.05 mg/L to about 10.0 mg/L vitamin B6;

from about 0.05 mg/L to about 12.0 mg/L vitamin B9; and

from about 0.05 mg/L to about 2.5 mg/L vitamin B12.

In a particular variation, a cell culture medium detailed herein comprises: (a) from about 300 mg/L (in some embodiments, 200 mg/L) to about 1200 mg/L cystine; (b) from about 0.05 mg/L to about 1.0 mg/L vitamin B2; (c) from about 0.05 mg/L to about 10.0 mg/L vitamin B6; (d) from about 0.05 mg/L to about 12.0 mg/L vitamin B9; and (e) from about 0.05 mg/L to about 2.5 mg/L vitamin B12, and where the cell culture medium (a) may in one variation be a CDM and/or (b) may further comprise one or more of the following components: (1) an iron source, such as ferric citrate or ferrous sulfate (which in one embodiment is present at a concentration of from about 2 μM to about 80 μM), and (2) hydrocortisone (which in one embodiment is present at a concentration from about 0.05 μM to about 0.25 μM).

[0008b] ~~In one aspect, there is provided~~Disclosed is a cell culture medium comprising:

from about 200 mg/L to about 1200 mg/L cystine;

from about 2 μM to about 80 μM ferric citrate; and

from about 0.05 μM to about 0.5 μM hydrocortisone.

acceptable color and where no more than 25% or 20% or 18% or 15% or 10% of the polypeptide product is a polypeptide charge variant (which in one embodiment is an acidic charge variant).

[0014] Compositions comprising the cell culture medium and one or more other components, such as a cell and/or a desired polypeptide (*e.g.*, an antibody), are also provided. The compositions encompass any and all phases of cell culture, such as seeding, cell growth, and cell production/maintenance.

[0014a] ~~In one aspect, there~~Disclosed is ~~provided~~ a composition comprising (a) a cell comprising an isolated nucleic acid encoding a polypeptide; and (b) a medium as described herein.

[0014b] ~~In one aspect, there~~Disclosed is ~~provided~~ a composition comprising:
(a) a polypeptide; and (b) a medium as described herein.

In one variation is provided a composition comprising: (a) a cell comprising an isolated nucleic acid encoding a polypeptide; and (b) a cell culture medium as provided herein. In another variation is provided a composition comprising: (a) a polypeptide; and (b) a cell culture medium as provided herein, where in one embodiment the polypeptide is secreted into the medium by a cell comprising an isolated nucleic acid encoding the polypeptide or is released into the medium by lysis of a cell comprising an isolated nucleic acid encoding the polypeptide. The cell of the composition may be any cell detailed herein (*e.g.*, a CHO cell) and the cell culture medium of the composition may be any medium detailed herein, the same as if each and every combination of cell and medium where specifically and individually listed. Likewise, the polypeptide of the composition may be any polypeptide detailed herein and the medium of the composition may be any medium detailed herein, the same as if each and every combination of polypeptide and medium where specifically and individually listed.

[0015] Methods of growing cells (*i.e.*, culturing cells) by contacting the cells with a cell culture medium as detailed herein are provided.

[0015a] ~~In one aspect, there is provided~~Disclosed is a method of culturing cells, comprising the step of contacting the cells with a cell culture medium comprising:
from about 200 mg/L to about 1200 mg/L cystine;
from about 0.05 mg/L to about 1.0 mg/L vitamin B2;
from about 0.05 mg/L to about 10.0 mg/L vitamin B6;

from about 0.05 mg/L to about 12.0 mg/L vitamin B9; and

from about 0.05 mg/L to about 2.5 mg/L vitamin B12.

In a particular variation of a method of

growing a cell (*i.e.*, culturing a cell), the cell culture medium is a CDM. In one variation, a method of growing a cell (*i.e.*, culturing a cell) comprises the step of contacting the cell with a cell culture medium comprising: (a) from about 300 mg/L (in some embodiments, 200 mg/L) to about 1200 mg/L cystine; (b) from about 0.05 mg/L to about 1.0 mg/L vitamin B2; (c) from about 0.05 mg/L to about 10.0 mg/L vitamin B6; (d) from about 0.05 mg/L to about 12.0 mg/L vitamin B9; and (e) from about 0.05 mg/L to about 2.5 mg/L vitamin B12, where the cell culture medium may further comprise one or more of the following components: (1) an iron source, such as ferric citrate or ferrous sulfate (which in one embodiment is present at a concentration of from about 2 μM to about 80 μM), and (2) hydrocortisone (which in one embodiment is present at a concentration of from about 0.05 μM to about 0.25 μM).

[0015b] ~~In one aspect, there is provided~~Disclosed is a method of culturing cells, comprising the step of contacting the cells with a cell culture medium comprising:

from about 200 mg/L to about 1200 mg/L cystine;

from about 2 μM to about 80 μM ferric citrate; and

from about 0.05 μM to about 0.5 μM hydrocortisone.

In another

variation, a method of growing a cell (*i.e.*, culturing a cell) is provided where the method

about 0.05 μM to about 0.5 μM hydrocortisone; from about 0.11 μM to about 0.72 μM vitamin B2; from about 4.5 μM to about 30.0 μM vitamin B6; from about 3.4 μM to about 22.0 μM vitamin B9; and from about 0.2 μM to about 1.5 μM vitamin B12, and where the cell culture medium may further comprise one or more of the following components: (1) vitamin B1 (which in one embodiment is present at a concentration from about 2.0 μM to about 14.0 μM), (2) vitamin B3 (which in one embodiment is present at a concentration from about 11.0 μM to about 72.0 μM), (3) vitamin B5 (which in one embodiment is present at a concentration from about 6.8 μM to about 44.0 μM), and (4) vitamin B7 (which in one embodiment is present at a concentration from about 0.02 μM to about 0.24 μM).

[0018] Methods of producing a polypeptide by growing in a cell culture medium (*i.e.*, culturing in a cell culture medium) a cell comprising an isolated nucleic acid encoding the polypeptide are also provided, wherein: (a) the cell expresses the polypeptide and (b) the cell culture medium comprises: (1) from about 300 mg/L (in some embodiments, 200 mg/L) to about 1200 mg/L cystine; (2) from about 0.05 mg/L to about 1.0 mg/L vitamin B2; (3) from about 0.05 mg/L to about 10.0 mg/L vitamin B6; (4) from about 0.05 mg/L to about 12.0 mg/L vitamin B9; and (5) from about 0.05 mg/L to about 2.5 mg/L vitamin B12, and where the cell culture medium may further comprise one or more of the following components: (A) an iron source, such as ferric citrate or ferrous sulfate (which in one embodiment is present at a concentration of from about 2 μM to about 80 μM) and (B) hydrocortisone (which in one embodiment is present at a concentration from about 0.05 μM to about 0.25 μM).

[0018a] In one aspect, there is provided a method of producing a polypeptide comprising the steps of (a) culturing in a cell culture medium a cell comprising an isolated nucleic acid encoding the polypeptide, wherein:

~~(a)~~ the cell culture medium comprises:

from ~~about~~ 200 mg/L to ~~about~~ 1200 mg/L cystine;

from ~~about~~ 0.05 mg/L to ~~about~~ 1.0 mg/L vitamin B2;

from ~~about~~ 0.05 mg/L to ~~about~~ 10.0 mg/L vitamin B6;

from ~~about~~ 0.05 mg/L to ~~about~~ 12.0 mg/L vitamin B9;

from ~~about~~ 0.05 mg/L to ~~about~~ 2.5 mg/L vitamin B12; and

from 2 μM to 80 μM ferric citrate and

~~(b)~~ the cell expresses the polypeptide; and

(b) purifying the polypeptide expressed by the cell, wherein if the purified polypeptide is concentrated to a concentration of at least 100 mg/mL, then the purified polypeptide has a

color that is lighter than a reference standard color value selected from the group consisting of B1, BY1, Y1, GY1, and R1 according to the European Pharmacopeia color standards.

[0018b] ~~In one aspect, there is provided~~Disclosed is a method of producing a polypeptide comprising the step of culturing in a cell culture medium a cell comprising an isolated nucleic acid encoding the polypeptide, wherein:

(a) the cell culture medium comprises:

from about 200 mg/L to about 1200 mg/L cystine;

from about 2 μM to about 80 μM ferric citrate; and

from about 0.05 μM to about 0.5 μM hydrocortisone; and

(b) the cell expresses the polypeptide.

In another variation, a method of producing a polypeptide by growing in a cell culture medium (*i.e.*, culturing in a cell culture medium) a cell comprising an isolated nucleic acid encoding the polypeptide is provided, wherein: (a) the cell expresses the polypeptide and (b) the cell culture medium comprises: (1) from about 300 mg/L (in some embodiments, 200 mg/L) to about 1200 mg/L cystine; (2) from about 2 μM to about 80 μM ferric citrate; and (3) from about 0.05 μM to about 0.5 μM hydrocortisone, where the cell culture medium may further comprise one or more of the following components: (A) vitamin B2 (which in one embodiment is present at a concentration of from about 0.05 mg/L to about 1.0 mg/L); (B) vitamin B6 (which in one embodiment is present at a concentration of from about 0.05 mg/L to about 10.0 mg/L); (C) vitamin B9 (which in one embodiment is present at a concentration of from about 0.05 mg/L to about 12.0 mg/L); and (D) vitamin B12 (which in one embodiment is present at a concentration of from about 0.05 mg/L to about 2.5 mg/L).

[0019] In another variation of the disclosure, a method of producing a polypeptide by growing in a cell culture medium (*i.e.*, culturing in a cell culture medium) a cell comprising an isolated nucleic acid encoding the polypeptide are also provided, wherein: (a) the cell expresses the polypeptide and (b) the cell culture medium comprises: (a) from about 0.8 mM (in some embodiments, 0.7 mM) to about 2.5 mM cystine; (b) from about 0.11 μM to about 0.72 μM vitamin B2; (c) from about 4.5 μM to about 30.0 μM vitamin B6; (c) from about 3.4 μM to about 22.0 μM vitamin B9; and (d) from about 0.2 μM to about 1.5 μM vitamin B12, and where the cell culture medium may further comprise one or more of the following components: (1) an iron source, such as ferric citrate or ferrous sulfate (which in one embodiment is present at a concentration of from about 11.0 μM to about 36.0 μM), (2) vitamin B1 (which in one embodiment is present at a concentration from about 2.0 μM to about 14.0 μM), (3) vitamin B3 (which in one embodiment is present at a concentration from about 11.0 μM to about 72.0 μM), (4) vitamin B5 (which in one embodiment is present at a concentration from about 6.8 μM to about 44.0 μM), and (5) vitamin B7 (which in one embodiment is present at a concentration from about 0.02 μM to about 0.24 μM).

[0020] In yet another variation of the disclosure, a method of producing a polypeptide by growing in a cell culture medium (*i.e.*, culturing in a cell culture medium) a cell comprising an isolated nucleic acid encoding the polypeptide are also provided, wherein: (a) the cell expresses the polypeptide and (b) the cell culture medium comprises: from about 0.7 mM to about 2.5 mM cystine; from about 2 μM to about 80 μM ferric citrate; from about 0.05 μM to about 0.5 μM hydrocortisone; from about 0.11 μM to about 0.72 μM vitamin B2; from about 4.5 μM to about 30.0 μM vitamin B6; from about 3.4 μM to about 22.0 μM vitamin B9; and from about 0.2 μM to about 1.5 μM vitamin B12, and where the cell culture medium may further comprise one or more of the following components: (1) vitamin B1 (which in one embodiment is present at a concentration from about 2.0 μM to about 14.0 μM), (2) vitamin B3 (which in one embodiment is present at a concentration from about 11.0 μM to about 72.0 μM), (3) vitamin B5 (which in one embodiment is present at a concentration from about 6.8 μM to about 44.0 μM), and (4) vitamin B7 (which in one embodiment is present at a concentration from about 0.02 μM to about 0.24 μM).

[0020a] ~~In one aspect, there is provided~~Disclosed is a polypeptide produced by the method as described herein.

VEGF, anti-mesothelin, anti-PCSK9 or anti-Beta7 antibody. Polypeptides produced according to the methods provided herein or present in the described compositions may be isolated from the cell culture medium and may be further purified. Polypeptides (such as antibodies) may also be concentrated to achieve a desirable concentration. Methods of concentrating polypeptides are known in the field, for example, the concentration of a polypeptide or protein may be increased by using ultrafiltration. In a particular variation, the polypeptide is isolated at a concentration of at least 100 mg/mL or 150 mg/mL and/or appears as a colorless or slightly colored liquid. In a particular variation, a composition comprises the isolated polypeptide at a concentration of at least 100 mg/mL and/or appears as a colorless or slightly colored liquid. In another variation, a composition comprises the isolated polypeptide at a concentration of at least 1 mg/mL or 10 mg/mL or 50 mg/mL or 75 mg/mL and/or appears as a colorless or slightly colored liquid. In another variation, a composition comprises the isolated polypeptide at a concentration of at least about any one of 1 mg/mL or 10 mg/mL or 50 mg/mL or 75 mg/mL and/or appears as a colorless or slightly colored liquid. In another variation, a composition comprises the isolated polypeptide at a concentration of at least about any one of 1 mg/mL or 10 mg/mL or 50 mg/mL or 75 mg/mL to about 125 mg/mL or to about 150 mg/mL and/or appears as a colorless or slightly colored liquid.

[0021a] ~~In one aspect, there is provided~~Disclosed is a pharmaceutical composition comprising a polypeptide as described herein and a pharmaceutically acceptable carrier. Also

described is a composition comprising a polypeptide obtained by a method detailed herein and a pharmaceutically acceptable carrier.

[0021b] ~~In one aspect, there is provided~~Disclosed is a kit for supplementing a cell culture medium with chemically defined constituents, the kit comprising:

cystine in an amount to provide from about 200 mg/L to about 1200 mg/L cystine in the cell culture medium;

vitamin B2 in an amount to provide from about 0.05 mg/L to about 1.0 mg/L vitamin B2 in the cell culture medium;

vitamin B6 in an amount to provide from about 0.05 mg/L to about 10.0 mg/L vitamin B6 in the cell culture medium;

vitamin B9 in an amount to provide from about 0.05 mg/L to about 12.0 mg/L vitamin B9 in

the cell culture medium; and

vitamin B12 in an amount to provide from about 0.05 mg/L to about 2.5 mg/L vitamin B12

[0022] A kit for supplementing a cell culture medium with chemically defined constituents is also described, the kit comprising: (a) cystine in an amount to provide from about 300 mg/L (in some embodiments, 200 mg/L) to about 1200 mg/L cystine in the cell culture medium; (b) vitamin B2 in an amount to provide from about 0.05 mg/L to about 1.0 mg/L vitamin B2 in the cell culture medium; (c) vitamin B6 in an amount to provide from about 0.05 mg/L to about 10.0 mg/L vitamin B6 in the cell culture medium; (d) vitamin B9 in an amount to provide from about 0.05 mg/L to about 12.0 mg/L vitamin B9 in the cell culture medium; and (e) vitamin B12 in an amount to provide from about 0.05 mg/L to about 2.5 mg/L vitamin B12 in the cell culture medium, where the kit may further comprise one or more of the following components (1) an iron source, such as ferric citrate or ferrous sulfate (which in one embodiment is in an amount to provide the iron source at a concentration of from about 2 μM to about 80 μM) and (2) hydrocortisone (which in one embodiment is in an amount to provide hydrocortisone at a concentration from about 0.05 μM to about 0.25 μM).

[0022a] ~~In one aspect, there is provided~~Disclosed is a kit for supplementing a cell culture medium with chemically defined constituents, the kit comprising:

cystine in an amount to provide from about 200 mg/L to about 1200 mg/L cystine in the cell culture medium;

ferric citrate in an amount to provide a concentration of from about 2 μM to about 80 μM ferric citrate in the cell culture medium; and

hydrocortisone in an amount to provide a concentration of from about 0.05 μM to about 0.5 μM hydrocortisone in the cell culture medium.

In another

CLAIMS

What is claimed is:

1. A method of producing a polypeptide comprising the steps of

(a) culturing in a cell culture medium a cell comprising an isolated nucleic acid encoding the polypeptide, wherein:

the cell culture medium comprises:

from 200 mg/L to 1200 mg/L cystine;

from 0.05 mg/L to 1.0 mg/L vitamin B2;

from 0.05 mg/L to 10.0 mg/L vitamin B6;

from 0.05 mg/L to 12.0 mg/L vitamin B9;

from 0.05 mg/L to 2.5 mg/L vitamin B12; and

from 2 μM to 80 μM ferric citrate and

the cell expresses the polypeptide; and

(b) purifying the polypeptide expressed by the cell, wherein if the purified polypeptide is concentrated to a concentration of at least 100 mg/mL, then the purified polypeptide has a color that is lighter than a reference standard color value selected from the group consisting of B1, BY1, Y1, GY1, and R1 according to the European Pharmacopeia color standards.

2. The method of claim 1, wherein the cell culture medium comprises:
from 0.7 mM to 2.5 mM cystine;
from 0.11 μM to 0.72 μM vitamin B2;
from 4.5 μM to 30.0 μM vitamin B6;
from 3.4 μM to 22.0 μM vitamin B9; and
from 0.2 μM to 1.5 μM vitamin B12.

3. The method of claim 1 or 2, wherein the cell culture medium further comprises any one or more of vitamin B1, vitamin B3, vitamin B5 and vitamin B7.

4. The method of claim 3, wherein the cell culture medium further comprises any one or more of:
from 2.0 μM to 14.0 μM vitamin B1;
from 11.0 μM to 72.0 μM vitamin B3;
from 6.8 μM to 44.0 μM vitamin B5; and
from 0.02 μM to 0.14 μM vitamin B7.

5. The method of any one of claims 1-4, wherein the cell culture medium comprises ferric citrate at a concentration of from 11.0 μM to 36.0 μM.

6. The method of any one of claims 1-5, wherein the cell culture medium further comprises hydrocortisone.

7. The method of claim 6, wherein the concentration of hydrocortisone in the cell culture medium is from 0.05 μM to 0.25 μM.

8. The method of any one of claims 1-7, wherein the cell culture medium is a chemically defined cell culture medium.

9. The method of any one of claims 1-7, wherein the cell culture medium is a chemically undefined cell culture medium.

10. The method of any one of claims 1-9, wherein the culturing is during the cell's growth phase.

11. The method of any one of claims 1-10, wherein the culturing is during the cell's production phase.

12. The method of claim 11, wherein the method further comprises a step of adding cysteine to the cell culture medium.

13. The method of claim 12, wherein the cysteine is added in an amount to provide from 80 mg/L to 1500 mg/L cysteine in the cell culture medium.

14. The method of claim 12, wherein the cysteine is added in an amount to provide 1500 mg/L cysteine in the cell culture medium.

15. The method of claim 12, wherein the cysteine is added in an amount to provide 140 mg/L cysteine in the cell culture medium.

16. The method of any one of claims 1-15, wherein the polypeptide is an antibody.

17. The method of claim 16, wherein the antibody is an IgG1 antibody.

18. The method of claim 16 or 17, wherein the antibody is an anti-VEGF, anti-mesothelin, anti-PCSK9 or anti-Beta7 antibody.

19. The method of claim 1, wherein if the purified polypeptide is concentrated to a concentration of at least 125 mg/mL, then the purified polypeptide has a color that is lighter than a reference standard color value selected from the group consisting of B1, BY1, Y1, GY1, and R1 according to the European Pharmacopeia color standards.

20. The method of claim 1, wherein if the purified polypeptide is concentrated to a concentration of at least 150 mg/mL, then the purified polypeptide has a color that is lighter than a reference standard color value selected from the group consisting of B1, BY1, Y1, GY1, and R1 according to the European Pharmacopeia color standards.

21. The method of claim 1, wherein if the purified polypeptide is concentrated to a concentration of at least 100 mg/mL, then the purified polypeptide has a color that is lighter than a reference standard color value selected from the group consisting of B2, BY2, Y2, GY2, and R2 according to the European Pharmacopeia color standards.

22. The method of claim 1, wherein if the purified polypeptide is concentrated to a concentration of at least 100 mg/mL, then the purified polypeptide has a color that is lighter than a reference standard color value selected from the group consisting of B3, BY3, Y3, GY3, and R3 according to the European Pharmacopeia color standards.

23. The method of claim 1, wherein the color of the purified polypeptide is determined by an assay selected from the group consisting of the COC assay, the Total Color assay, and the NIFTY assay.

24. The method of claim 1, further comprising (c) concentrating the purified polypeptide in step (b) to a concentration of at least 100 mg/mL.

25. The method of claim 24, wherein the purified polypeptide is concentrated to a concentration of at least 125 mg/mL.

26. The method of claim 24, wherein the purified polypeptide is concentrated to a concentration of at least 150 mg/mL.

CLAIMS

What is claimed is:

1. A method of culturing cells, comprising the step of contacting the cells with a cell culture medium comprising:
from about 200 mg/L to about 1200 mg/L cystine;
from about 0.05 mg/L to about 1.0 mg/L vitamin B2;
from about 0.05 mg/L to about 10.0 mg/L vitamin B6;
from about 0.05 mg/L to about 12.0 mg/L vitamin B9; and
from about 0.05 mg/L to about 2.5 mg/L vitamin B12.

2. The method of claim 1, comprising the step of contacting the cells with a cell culture medium comprising:
from about 0.7 mM to about 2.5 mM cystine;
from about 0.11 μM to about 0.72 μM vitamin B2;
from about 4.5 μM to about 30.0 μM vitamin B6;
from about 3.4 μM to about 22.0 μM vitamin B9; and
from about 0.2 μM to about 1.5 μM vitamin B12.

3. The method of claim 1 or 2, wherein the cell culture medium further comprises any one or more of vitamin B1, vitamin B3, vitamin B5 and vitamin B7.

4. The method of claim 3, wherein the cell culture medium further comprises any one or more of:
from about 2.0 μM to about 14.0 μM vitamin B1;
from about 11.0 μM to about 72.0 μM vitamin B3;
from about 6.8 μM to about 44.0 μM vitamin B5; and
from about 0.02 μM to about 0.14 μM vitamin B7.

5. The method of any one of claims 1-4, wherein the cell culture medium further comprises an iron source.

6. The method of claim 5, wherein the iron source is ferric citrate or ferrous sulfate.

7. The method of any one of claims 1-6, wherein the cell culture medium comprises ferric citrate at a concentration of from about 2 μM to about 80 μM.

8. The method of any one of claims 1-7, wherein the cell culture medium comprises ferric citrate at a concentration of from about 11.0 μM to about 36.0 μM.

9. The method of any one of claims 1-8, wherein the cell culture medium further comprises hydrocortisone.

10. The method of claim 9, wherein the concentration of hydrocortisone in the cell culture medium is from about 0.05 μM to about 0.25 μM.

11. The method of any one of claims 1-10, wherein the cell culture medium is a chemically defined cell culture medium.

12. The method of any one of claims 1-10, wherein the cell culture medium is a chemically undefined cell culture medium.

13. The method of any one of claims 1-12, wherein the cells are contacted with the cell culture medium during the cells' growth phase.

14. The method of any one of claims 1-13, wherein the cells are contacted with the cell culture medium during the cells' production phase.

15. The method of claim 14, wherein the method further comprises a step of adding cysteine to the cell culture medium.

16. The method of claim 15, wherein the cysteine is added in an amount to provide from about 80 mg/L to about 1500 mg/L cysteine in the cell culture medium.

17. The method of claim 15, wherein the cysteine is added in an amount to provide about 1500 mg/L cysteine in the cell culture medium.

18. The method of claim 15, wherein the cysteine is added in an amount to provide about 140 mg/L cysteine in the cell culture medium.

19. A method of producing a polypeptide comprising the step of culturing in a cell culture medium a cell comprising an isolated nucleic acid encoding the polypeptide, wherein:
(a) the cell culture medium comprises:
from about 200 mg/L to about 1200 mg/L cystine;
from about 0.05 mg/L to about 1.0 mg/L vitamin B2;
from about 0.05 mg/L to about 10.0 mg/L vitamin B6;
from about 0.05 mg/L to about 12.0 mg/L vitamin B9;
from about 0.05 mg/L to about 2.5 mg/L vitamin B12; and

(b) the cell expresses the polypeptide.

20. The method of claim 19, wherein the cell culture medium comprises:
from about 0.7 mM to about 2.5 mM cystine;
from about 0.11 μM to about 0.72 μM vitamin B2;
from about 4.5 μM to about 30.0 μM vitamin B6;
from about 3.4 μM to about 22.0 μM vitamin B9; and
from about 0.2 μM to about 1.5 μM vitamin B12.

21. The method of claim 19 or 20, wherein the cell culture medium further comprises any one or more of vitamin B1, vitamin B3, vitamin B5 and vitamin B7.

22. The method of claim 21, wherein the cell culture medium further comprises any one or more of:
from about 2.0 μM to about 14.0 μM vitamin B1;
from about 11.0 μM to about 72.0 μM vitamin B3;
from about 6.8 μM to about 44.0 μM vitamin B5; and
from about 0.02 μM to about 0.14 μM vitamin B7.

23. The method of any one of claims 19-22, wherein the cell culture medium further comprises an iron source.

24. The method of claim 23, wherein the iron source is ferric citrate or ferrous sulfate.

25. The method of any one of claims 19-24, wherein the cell culture medium comprises ferric citrate at a concentration of from about 2 μM to about 80 μM.

26. The method of any one of claims 19-25, wherein the cell culture medium comprises ferric citrate at a concentration of from about 11.0 μM to about 36.0 μM.

27. The method of any one of claims 19-26, wherein the cell culture medium further comprises hydrocortisone.

28. The method of claim 27, wherein the concentration of hydrocortisone in the cell culture medium is from about 0.05 μM to about 0.25 μM.

29. The method of any one of claims 19-28, wherein the cell culture medium is a chemically defined cell culture medium.

30. The method of any one of claims 19-28, wherein the cell culture medium is a chemically undefined cell culture medium.

31. The method of any one of claims 19-30, wherein the culturing is during the cell's growth phase.

32. The method of any one of claims 19-31, wherein the culturing is during the cell's production phase.

33. The method of claim 32, wherein the method further comprises a step of adding cysteine to the cell culture medium.

34. The method of claim 33, wherein the cysteine is added in an amount to provide from about 80 mg/L to about 1500 mg/L cysteine in the cell culture medium.

35. The method of claim 33, wherein the cysteine is added in an amount to provide about 1500 mg/L cysteine in the cell culture medium.

36. The method of claim 33, wherein the cysteine is added in an amount to provide about 140 mg/L cysteine in the cell culture medium.

37. The method of any one of claims 19-36, wherein the polypeptide is an antibody.

38. The method of claim 37, wherein the antibody is an IgG1 antibody.

39. The method of claim 37 or 38, wherein the antibody is an anti-VEGF, anti-mesothelin, anti-PCSK9 or anti-Beta7 antibody.

40. The method of any one of claims 19-39, further comprising the step of isolating the polypeptide from the cell culture medium.

41. The method of claim 40, wherein a composition comprising the isolated polypeptide appears as a colorless or slightly colored liquid.

42. The method of claim 41, wherein the composition comprises the isolated polypeptide at a concentration of at least 100 mg/mL.

43. A polypeptide produced by the method of any one of claims 19-42.

44. A pharmaceutical composition comprising a polypeptide of claim 43 and a pharmaceutically acceptable carrier.

45. A kit for supplementing a cell culture medium with chemically defined constituents, the kit comprising:
cystine in an amount to provide from about 200 mg/L to about 1200 mg/L cystine in the cell culture medium;
vitamin B2 in an amount to provide from about 0.05 mg/L to about 1.0 mg/L vitamin B2 in the cell culture medium;
vitamin B6 in an amount to provide from about 0.05 mg/L to about 10.0 mg/L vitamin B6 in the cell culture medium;
vitamin B9 in an amount to provide from about 0.05 mg/L to about 12.0 mg/L vitamin B9 in the cell culture medium; and
vitamin B12 in an amount to provide from about 0.05 mg/L to about 2.5 mg/L vitamin B12.

46. The kit of claim 45, the kit comprising:
cystine in an amount to provide from about 0.7 mM to about 2.5 mM cystine in the cell culture medium;
vitamin B2 in an amount to provide from about 0.11 μM to about 0.72 μM vitamin B2 in the cell culture medium;
vitamin B6 in an amount to provide from about 4.5 μM to about 30.0 μM vitamin B6 in the cell culture medium;
vitamin B9 in an amount to provide from about 3.4 μM to about 22.0 μM vitamin B9 in the cell culture medium; and
vitamin B12 in an amount to provide from about 0.2 μM to about 1.5 μM vitamin B12 in the cell culture medium.

47. The kit of claim 45 or 46, wherein the kit further comprises any one or more of vitamin B1, vitamin B3, vitamin B5 and vitamin B7.

48. The kit of claim 47, wherein the kit further comprises any one or more of:
vitamin B1 in an amount to provide from about 2.0 μM to about 14.0

μM vitamin B1 in the cell culture medium;
vitamin B3 in an amount to provide from about 11.0 μM to about 72.0 μM vitamin B3 in the cell culture medium;
vitamin B5 in an amount to provide from about 6.8 μM to about 44.0 μM vitamin B5 in the cell culture medium; and
vitamin B7 in an amount to provide from about 0.02 μM to about 0.14 μM vitamin B7 in the cell culture medium.

49. The kit of any one of claims 45-48, wherein the kit further comprises an iron source.

50. The kit of claim 49, wherein the iron source is ferric citrate or ferrous sulfate.

51. The kit of any one of claims 45-50, wherein the kit further comprises hydrocortisone.

52. A cell culture medium comprising:
from about 200 mg/L to about 1200 mg/L cystine;
from about 0.05 mg/L to about 1.0 mg/L vitamin B2;
from about 0.05 mg/L to about 10.0 mg/L vitamin B6;
from about 0.05 mg/L to about 12.0 mg/L vitamin B9; and
from about 0.05 mg/L to about 2.5 mg/L vitamin B12.

53. The medium of claim 52, comprising:
from about 0.7 mM to about 2.5 mM cystine;
from about 0.11 μM to about 0.72 μM vitamin B2;
from about 4.5 μM to about 30.0 μM vitamin B6;
from about 3.4 μM to about 22.0 μM vitamin B9; and
from about 0.2 μM to about 1.5 μM vitamin B12.

54. The medium of claim 52 or 53, further comprising any one or more of vitamin B1, vitamin B3, vitamin B5 and vitamin B7.

55. The medium of claim 54, further comprising any one or more of:
from about 2.0 μM to about 14.0 μM vitamin B1;
from about 11.0 μM to about 72.0 μM vitamin B3;
from about 6.8 μM to about 44.0 μM vitamin B5; and
from about 0.02 μM to about 0.14 μM vitamin B7.

56. The medium of any one of claims 52-55, wherein the cell culture medium further comprises an iron source.

57. The medium of claim 56, wherein the iron source is ferric citrate or ferrous sulfate.

58. The medium of claim 57, wherein the cell culture medium comprises ferric citrate at a concentration of from about 2 μM to about 80 μM.

59. The medium of claim 58, wherein the cell culture medium comprises ferric citrate at a concentration of from about 11.0 μM to about 36.0 μM.

60. The medium of any one of claims 52-59, wherein the cell culture medium further comprises hydrocortisone.

61. The medium of claim 60, wherein the cell culture medium comprises hydrocortisone at a concentration of from about 0.05 μM to about 0.25 μM.

62. A method of culturing cells, comprising the step of contacting the cells with a cell culture medium comprising:
from about 200 mg/L to about 1200 mg/L cystine;
from about 2 μM to about 80 μM ferric citrate; and
from about 0.05 μM to about 0.5 μM hydrocortisone.

63. The method of claim 62, wherein the cell culture medium further comprises vitamin B2, vitamin B6, vitamin B9 and/or vitamin B12.

64. The method of claim 62 or 63, wherein the cell culture medium comprises from about 0.05 mg/L to about 1.0 mg/L of vitamin B2.

65. The method of any one of claims 62-64, wherein the cell culture medium comprises from about 0.05 mg/L to about 10.0 mg/L of vitamin B6.

66. The method of any one of claims 62-65, wherein the cell culture medium comprises from about 0.05 mg/L to about 12.0 mg/L of vitamin B9.

67. The method of any one of claims 62-66, wherein the cell culture medium comprises from about 0.05 mg/L to about 2.5 mg/L of vitamin B12.

68. The method of any one of claims 62-67, wherein the cell culture medium is a chemically defined cell culture medium.

69. The method of any one of claims 62-67, wherein the cell culture medium is a chemically undefined cell culture medium.

70. The method of any one of claims 62-69, wherein the cells are contacted with the cell culture medium during the cells' growth phase.

71. The method of any one of claims 62-70, wherein the cells are contacted with the cell culture medium during the cells' production phase.

72. The method of claim 71, wherein the method further comprises a step of adding cysteine to the cell culture medium.

73. The method of claim 72, wherein the cysteine is added in an amount to provide from about 80 mg/L to about 1500 mg/L cysteine in the cell culture medium.

74. The method of claim 72, wherein the cysteine is added in an amount to provide about 1500 mg/L cysteine in the cell culture medium.

75. The method of claim 72, wherein the cysteine is added in an amount to provide about 140 mg/L cysteine in the cell culture medium.

76. A method of producing a polypeptide comprising the step of culturing in a cell culture medium a cell comprising an isolated nucleic acid encoding the polypeptide, wherein:
(a) the cell culture medium comprises:
from about 200 mg/L to about 1200 mg/L cystine;
from about 2 μM to about 80 μM ferric citrate; and
from about 0.05 μM to about 0.5 μM hydrocortisone; and

(b) the cell expresses the polypeptide.

77. The method of claim 76, wherein the cell culture medium further comprises vitamin B2, vitamin B6, vitamin B9 and/or vitamin B12.

78. The method of claim 76 or 77, wherein the cell culture medium comprises from about 0.05 mg/L to about 1.0 mg/L of vitamin B2.

79. The method of any one of claims 76-78, wherein the cell culture medium comprises from about 0.05 mg/L to about 10.0 mg/L of vitamin B6.

80. The method of any one of claims 76-79, wherein the cell culture medium comprises from about 0.05 mg/L to about 12.0 mg/L of vitamin B9.

81. The method of any one of claims 76-80, wherein the cell culture medium comprises from about 0.05 mg/L to about 2.5 mg/L of vitamin B12.

82. The method of any one of claims 76-81, wherein the cell culture medium is a chemically defined cell culture medium.

83. The method of any one of claims 76-81, wherein the cell culture medium is a chemically undefined cell culture medium.

84. The method of any one of claims 76-83, wherein the culturing is during the cell's growth phase.

85. The method of any one of claims 76-84, wherein the culturing is during the cell's production phase.

86. The method of claim 85, wherein the method further comprises a step of adding cysteine to the cell culture medium.

87. The method of claim 86, wherein the cysteine is added in an amount to provide from about 80 mg/L to about 1500 mg/L cysteine in the cell culture medium.

88. The method of claim 86, wherein the cysteine is added in an amount to provide about 1500 mg/L cysteine in the cell culture medium.

89. The method of claim 86, wherein the cysteine is added in an amount to provide about 140 mg/L cysteine in the cell culture medium.

90. The method of any one of claims 76-89, wherein the polypeptide is an antibody.

91. The method of claim 90, wherein the antibody is an IgG1 antibody.

92. The method of claim 90 or 91, wherein the antibody is an anti-VEGF, anti-mesothelin, anti-PCSK9 or anti-Beta7 antibody.

93. The method of any one of claims 76-92, further comprising the step of isolating the polypeptide from the cell culture medium.

94. The method of claim 93, wherein a composition comprising the isolated polypeptide appears as a colorless or slightly colored liquid.

95. The method of claim 94, wherein the composition comprises the isolated polypeptide at a concentration of at least 100 mg/mL.

96. A polypeptide produced by the method of any one of claims 76-95.

97. A pharmaceutical composition comprising a polypeptide of claim 96 and a pharmaceutically acceptable carrier.

98. A kit for supplementing a cell culture medium with chemically defined constituents, the kit comprising:
cystine in an amount to provide from about 200 mg/L to about 1200 mg/L cystine in the cell culture medium;
ferric citrate in an amount to provide a concentration of from about 2 μM to about 80 μM ferric citrate in the cell culture medium; and
hydrocortisone in an amount to provide a concentration of from about 0.05 μM to about 0.5 μM hydrocortisone in the cell culture medium.

99. The kit of claim 98, wherein the kit further comprises vitamin B2, vitamin B6, vitamin B9 and/or vitamin B12.

100. The kit of claim 98 or 99, wherein the kit comprises vitamin B2 in an amount to provide from about 0.05 mg/L to about 1.0 mg/L of vitamin B2 in the cell culture medium.

101. The kit of any one of claims 98-100, wherein the kit comprises vitamin B6 in an amount to provide from about 0.05 mg/L to about 10.0 mg/L of vitamin B6 in the cell culture medium.

102. The kit of any one of claims 98-101, wherein the kit comprises vitamin B9 in an amount to provide from about 0.05 mg/L to about 12.0 mg/L of vitamin B9 in the cell culture medium.

103. The kit of any one of claims 98-102, wherein the kit comprises vitamin B12 in an amount to provide from about 0.05 mg/L to about 2.5 mg/L of vitamin B12 in the cell culture medium.

104. A cell culture medium comprising:
from about 200 mg/L to about 1200 mg/L cystine;
from about 2 μM to about 80 μM ferric citrate; and
from about 0.05 μM to about 0.5 μM hydrocortisone.

105. The medium of claim 104, wherein the medium further comprises vitamin B2, vitamin B6, vitamin B9 and/or vitamin B12.

106. The medium of claim 104 or 105, wherein the medium comprises from about 0.05 mg/L to about 1.0 mg/L of vitamin B2.

107. The medium of any one of claims 104-106, wherein the medium comprises from about 0.05 mg/L to about 10.0 mg/L of vitamin B6.

108. The medium of any one of claims 104-107, wherein the medium comprises from about 0.05 mg/L to about 12.0 mg/L of vitamin B9.

109. The medium of any one of claims 104-108, wherein the medium comprises from about 0.05 mg/L to about 2.5 mg/L of vitamin B12.

110. A composition comprising (a) a cell comprising an isolated nucleic acid encoding a polypeptide; and (b) a medium according to any one of claims 52-61 and 104-109.

111. A composition comprising: (a) a polypeptide; and (b) a medium according to any one of claims 52-61 and 104-109.