

What is claimed is:

1. A cell culture medium for eukaryotic cells expressing Aflibercept, comprising:

a basal medium or a feed medium; and

5-methylthioadenosine ~~or nicotinamide~~.

2. The cell culture medium of claim 1, wherein a concentration of 5-methylthioadenosine in the cell culture medium is at least ~~about~~ 10 nM.

~~3. The cell culture medium of claim 1, wherein a concentration of the nicotinamide in the cell culture medium is at least about 50 nM.~~

34. The cell culture medium of claim 1 further comprising one or more acids selected from lactic acid, phenyl lactic acid, indolelactic acid, succinic acid, alpha-hydroxyisovaleric acid, alpha-hydroxyisocaproic acid, 2-(4-hydroxyphenyl)lactic acid, or 2-hydroxy-3-methylvaleric acid, salts of these acids, esters of these acids and combinations thereof.

45. The cell culture medium of claim 2, wherein a titer of the Aflibercept produced in the cell culture medium is at least ~~about~~ 2% greater than another cell culture medium that does not have at least ~~about~~ 10 nM 5-methylthioadenosine.

~~6. The cell culture medium of claim 3, wherein a titer of the Aflibercept produced in the cell culture medium is at least about 2% greater than another cell culture medium that does not have at least about 50 nM nicotinamide.~~

57. The cell culture medium of claim 1, wherein the 5-methylthioadenosine ~~or the nicotinamide~~ can be in the form of its salts or esters.

~~68.~~ The cell culture medium of claim 1, wherein pH of the medium is maintained in the range of ~~about~~ 6.5 to ~~about~~ 8.

79. The cell culture medium of claim 1, wherein the medium does not have a protein derived from an animal.

810. The cell culture medium of claim 1, wherein the medium is a serum-free medium.

911. The cell culture medium of claim 1, wherein the medium is a chemically-defined medium.

1012. A method for culturing eukaryotic cells for increasing production of Aflibercept, comprising the steps of:

culturing eukaryotic cells in a cell culture medium;

supplementing the cell culture medium with 5-methylthioadenosine, wherein concentration of the 5-methylthioadenosine is ~~about~~ 10 nM to ~~about~~ 200 nM; and

producing Aflibercept in the eukaryotic cells,

wherein the supplementation with 5-methylthioadenosine increases a titer of the Aflibercept.

~~1113.~~ The method of claim ~~1012~~, wherein the titer of the Aflibercept is at least ~~about~~ 2% greater than another method with a cell culture medium that does not have at least ~~about~~ 10 nM 5-methylthioadenosine.

~~1214.~~ The method of claim ~~1012~~, wherein the cell culture medium further comprises one or more acids selected from lactic acid, phenyl lactic acid, indolelactic acid, succinic acid, alpha-hydroxyisovaleric acid, alpha-hydroxyisocaproic acid, 2-(4-hydroxyphenyl)lactic acid, or 2-hydroxy-3-methylvaleric acid, salts of these acids, esters of these acids and combinations thereof.

~~1315.~~ The method of claim ~~1012~~, wherein the eukaryotic cells include at least one selected from the group consisting of: Baby Hamster Kidney cell lines, Chinese Hamster Ovary cell lines, Murine myeloma cell lines, Mouse myeloma cell lines, Human embryonic kidney cell lines, Human-retina-derived cell lines, and Amniocyte cell lines.

~~1416.~~ The method of claim ~~1012~~, wherein the Aflibercept is secreted in the medium.

~~1517.~~ The method of claim ~~1012~~, wherein the cell culture medium does not have a protein derived from an animal.

~~1618.~~ The method of claim ~~1012~~, wherein the cell culture medium is a serum-free medium.

~~1719.~~ The method of claim ~~1012~~, wherein the cell culture medium is a chemically-defined medium.

~~20.—A method for culturing eukaryotic cells for increasing production of Aflibercept, comprising the steps of:~~

~~culturing cells in a defined cell culture medium;~~

~~supplementing the cell culture medium with nicotinamide, wherein concentration of the nicotinamide is about 50 nM to about 2000 nM; and~~

~~producing Aflibercept in the eukaryotic cells, wherein the supplementation with nicotinamide increases a titer of the Aflibercept.~~

21. — ~~The method of claim 20, wherein the titer of the Aflibercept is at least about 2% greater than another method with a cell culture medium that does not have at least about 50 nM nicotinamide.~~

22. — ~~The method of claim 20, wherein the cell culture medium further comprises one or more acids selected from lactic acid, phenyl lactic acid, indolelactic acid, succinic acid, alpha-hydroxyisovaleric acid, alpha-hydroxyisocaproic acid, 2-(4-hydroxyphenyl)lactic acid, or 2-hydroxy-3-methylvaleric acid, salts of these acids, esters of these acids and combinations thereof.~~

23. — ~~The method of claim 20, wherein the eukaryotic cells include at least one selected from the group consisting of: Baby Hamster Kidney cell lines, Chinese Hamster Ovary cell lines, Murine myeloma cell lines, Mouse myeloma cell lines, Human embryonic kidney cell lines, Human retina derived cell lines, and Amniocyte cell lines.~~

24. — ~~The method of claim 20, wherein the Aflibercept is secreted in the medium.~~

25. — ~~The method of claim 20, wherein the cell culture medium does not have a protein derived from an animal.~~

26. — ~~The method of claim 20, wherein the cell culture medium is a serum-free medium.~~

27. — ~~The method of claim 20, wherein the cell culture medium is a chemically-defined medium.~~

1828. A method for producing Aflibercept, comprising:

introducing into a cell a nucleic acid comprising a nucleotide sequence encoding Aflibercept;

culturing the cell in a cell culture medium comprising ~~at least about 50 nM nicotinamide or~~ at least ~~about~~ 10 nM 5-methylthioadenosine; and

producing the Aflibercept in the cell.