

CLAIMS

1. A method for obtaining stable high producer cell clones from myeloma cell lines in protein-free medium producing recombinant antibodies for industrial purposes that comprise three stages:

- I. Adaptation to protein-free medium by a stepwise reduction of a lipid-enriched supplement to chemically defined medium in low density stationary cell culture
- II. Adaptation to protein-free medium in high density cell culture using perfusion fermentation system at lab scale
- III. Selection of stable high producer cell clones from cells at the end of fermentation

2. The method of claim 1 wherein the first stage comprises the following steps:

- I. Adaptation to grow in PFHMII cell culture medium + 3,5 g/L Cell Boost. Serial passage is performed until Xv reach a constant value.
- II. Adaptation to grow in PFHMII cell culture medium + 1 g/L Cell Boost. Serial passage is performed until Xv reach a constant value.
- III. Adaptation to grow in PFHMII cell culture medium. Cells are allowed to grow in protein-free medium without any supplement for 60 days.

3. The method of claim 1 wherein the second stage comprises the following steps:

- I. Adaptation to grow in PFHMII cell culture medium at high cell density ($5 - 10 \times 10^6$ cells/ml) using perfusion fermentation system in 5 L bioreactor for more than 21 days.
- II. Cells at the end of fermentation are frozen and storage in liquid nitrogen (end production cell bank, EPCB)

4. The method of claim 1 wherein the third stage comprises the following steps:

- I. Defrosted cells from EPCB are subjected to cell cloning by the limiting dilution method
- II. Antibody-secreting clones are detected by a highly sensitive and specific ELISA method using an anti-idiotypic antibody as capture antigen
- III. Quantification of the antibody-producer cell sub-population in selected clones by FACS using intracellular immunoglobulin staining
- IV. Kinetic studies to select clones with higher specific growth rate (μ) and specific production rate (QP).

5. The myeloma cell line obtained by the method of any of the claims 1 to 4.
6. The cell line according to claim 5 wherein the myeloma is the NS0 cell line.
7. The NS0 cell line according to claim 6 wherein contains a sequence encoding for the humanized recombinant antibody anti-NeuGcGM3 14F7h.
8. The humanized recombinant antibody anti-NeuGcGM3 14F7h secreted by the cell lines obtained by the method of claim 6.
9. The humanized recombinant antibody anti-NeuGcGM3 14F7h secreted by the cell line according to claim 7 wherein said cell line is the clone B58-31E7
10. The humanized recombinant antibody anti-NeuGcGM3 14F7h according to claim 8 wherein said humanized recombinant antibody anti-NeuGcGM3 14F7h has the following molecular phenotype:

Identity attributes	Molecular features	Data
Primary structure	Molecular Mass	Heavy Chain: ≈ 50770 Da Light Chain: ≈ 23665 Da
	Peptide mapping	Profile Conform to Example
High order structure	Secondary structure	CD Profile Conform to Example
	Tertiary structure	Max emission $\approx 333\text{nm}$ Absorbance Ratio $_{330\text{nm}/350\text{nm}} \approx 1.23$
Glycosylation	HPLC Profiling	G0F% $=33.1 \pm 11.7$ G1F% $=42.0 \pm 7.5$ G2F% $=10.9 \pm 5.1$ Fuc% $=98.4 \pm 0.6$ Sial% $=7.2 \pm 4.2$
Heterogeneity	Charges	Main Peak: $96.0 \pm 1.8 \%$
	Aggregation	Main Peak: $99.8 \pm 0.3 \%$
Function	Antigen recognition	EC ₅₀ = $0.245 \pm 0.180\text{ug/mL}$

11. The humanized recombinant antibody anti-NeuGcGM3 14F7h according to claim 9 having *in vitro* and *in vivo* anti-tumor activity.