EXHIBIT 5

IN THE FEDERAL COURT
OF AUSTRALIA
VICTORIA DISTRICT REGISTRY
GENERAL DIVISION

No. VG 868 of 1995

On Appeal from the Commissioner of Patents

BETWEEN:

GENETICS INSTITUTE, INC.
Applicant

-and-

KIRIN-AMGEN, INC.
Respondent

KIRIN-AMGEN, INC.
Cross-Applicant

-and-

GENETICS INSTITUTE, INC.
Cross-Respondent

EXHIBIT NOTE

This is the exhibit marked **"JKB-3"** produced and shown me **JEFFREY K. BROWNE** at the time of swearing his affidavit on the 27 day of February 1997.

Jeffrey K. Browne

Sworn and subscribed before me
on this 27th day of February 1997:

Notary Public

MARY KENNEY
Commission # 1096859
Notary Public — California
Ventura County
My Comm. Expires May 5, 2000

AM 67 018641
CONFIDENTIAL
SUBJECT TO PROTECTIVE ORDER

Exhibit JKB-3

DECLARATION OF THOMAS W. STRICKLAND

I, Thomas W. Strickland, solemnly declare as follows:

1. I make this declaration in support of the position of Cilag GmbH opposing EP 411 678 entitled "Method for the Production of Erythropoietin" to Genetics Institute.

2. I have been employed by Amgen Inc. located in Thousand Oaks, California since April 1984. I currently hold the position of Research Scientist. I received my doctorate degree (Ph.D.) in Biochemistry from Vanderbilt University in 1981 and did my thesis on glycoprotein hormones. From February 1981 to March 1984, I held the position of Assistant Research Biological Chemist, Department of Biological Chemistry, University of California, Los Angeles (UCLA). My research activities at UCLA included studies on biosynthesis and protein folding of glycoprotein hormones; chemical modification, isolation and receptor cross-linking of glycoprotein hormones. A copy of my curriculum vitae is attached as Exhibit A.

3. I have read the claims of EP 411 678 and particularly Claims 8-11 which cover recombinant human erythropoietin glycosylation.

4. During 1985, my responsibilities at Amgen included involvement in the production of erythropoietin ("EPO"). As such, I am familiar with the steps of the manufacturing process used by Amgen in 1985 to produce erythropoietin. The erythropoietin manufactured for sale by Amgen in 1985 was produced according to the procedures of Example 10 of EP 148 605. These procedures for producing recombinant human erythropoietin can generally be described by the steps of:

(a) culturing in a suitable medium CHO cells containing a DNA sequence encoding human erythropoietin, said DNA sequence operatively linked to an expression control sequence, and

(b) recovering and separating the EPO from the cells and the medium.

as recited in Claim 8 of EP 411 678. Specifically, I was involved in the purification of bulk lots of erythropoietin prior to formulation. One of the lots which I purified in 1985 was designated L07B. This lot was purified according to the procedures disclosed in my patent, U.S. Patent No. 4,667,016.

5. Amgen's erythropoietin sold in 1985 also included O-linked glycosylation as recited in Claim 8. To verify this point, I conducted an analysis of a sample of rHuEPO lot L07B preserved in storage at Amgen since 1985. This sample was labelled L07B and had been maintained in a freezer at -70 °C since its preparation.

6. An aliquot of rHuEPO lot L07B was solvent exchanged into water using a Centricon ultrafiltration device (Amicon, Danvers, MA) by concentrating the solution and then reconstituting it with water repeatedly. The final concentrate was diluted to an A_{280} of 1.0 with a buffer consisting of 15 mM sodium phosphate pH 7.0, 5 mM Chaps, and 2.5 mM sodium azide (phosphate/Chaps/azide buffer).

7. Samples 1-8 were prepared as per the following table:

SAMPLE #	ul EPO	ul N-Glycanase	ul Sialidase	ul O-Glycanase	ul Buffer	Total Volume
1	10.0	0.0	0.0	0.0	10.0	20.0
2	10.0	0.0	0.0	0.0	10.0	20.0
3	3.0	1.0	0.0	0.0	18.0	20.0
4	3.0	1.0	1.0	0.0	15.0	20.0
5	3.0	1.0	1.0	2.0	13.0	20.0
6	0.0	1.0	0.0	0.0	19.0	20.0
7	0.0	1.0	1.0	0.0	18.0	20.0
8	0.0	1.0	1.0	2.0	16.0	20.0

using the following reagents:

1) EPO - the A_{280} = 1.0 sample of lot L07B in phosphate/Chaps/azide buffer.

2) N-Glycanase - Natural source N-Glycanase enzyme from Genzyme, lot #B1384.

3) Sialidase - Lyophilized Arthrobacter ureafaciens neuraminidase, Lot #123791, purchased from Calbiochem, reconstituted to 1 U/mL in phosphate/Chaps/azide buffer.

4) O-Glycanase - O-Glycanase, lot #11760 purchased from Genzyme. The 970 mU/mL stock was diluted to 100 mU/mL with phosphate/Chaps/azide buffer prior to use.

5) Buffer - phosphate/Chaps/azide buffer.

8. The resulting mixtures, samples 2-8, were incubated for approximately 22 hr at 37°C, while sample #1 was held at 4°C for this time. The samples were then prepared for polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE) by adding an equal volume of SDS sample buffer containing 10% mercaptoethanol and heating the samples at 65°C for 5 minutes. The samples were run on a 12.5% acrylamide gel, as shown in Exhibit B, with lane #1 containing 0.5 µL of Bio-Rad low range molecular weight markers, and lanes 2-9 containing samples 1-8. The gel was stained with Coomassie blue to visualize the protein bands. A photocopy of the gel is shown in Exhibit B.

9. The N-Glycanase and O-Glycanase used in the analysis were the same as that available in 1985 from Genzyme Corporation located in Boston, Massachusetts. I attached as Exhibit C product literature received at Amgen in 1985 showing the availability of N-Glycanase and O-Glycanase. The sialidase used was commercially available

from Calbiochem in 1985. The techniques of analysis described above were also known to those skilled in the art in 1985.

10. The results of the analysis on the Amgen rHuEPO lot LO7B clearly show the presence of O-linked glycosylation. As shown by the photograph in Exhibit B, there is a decrease in molecular weight of the rHuEPO sample upon treatment with N-Glycanase (Lane 4) which removes the N-linked oligosaccharides, and a further decrease upon treatment with sialidase to remove sialic acid from the O-linked oligosaccharide (Lane 5). A further decrease in molecular weight is visible after treating the sample with O-Glycanase to remove the O-linked disaccharide gal ß(1-3) galNAc (Lane 6). This decrease in molecular weight confirms the existence of O-linked glycosylation in rHuEPO lot L07B.

11. The decrease in molecular weight seen upon treatment with O-Glycanase as described above and the specificity of O-Glycanase for the gal ß(1-3)galNAc core disaccharide as described in the product data sheet in Exhibit C demonstrates the presence of N-acetylgalactosamine as recited in Claim 11 of EP 411 678. The presence of N-acetylgalactosamine on rHuEPO is further shown by direct analysis of the monosaccharide composition of Amgen produced rHuEPO following enzymatic removal of the N-linked carbohydrate. These results are reported in Browne, et al., Cold Spring Harbor Symposia on Quantitative Biology, 51, 693-702 (1986).

12. Further analysis of the monosaccharide content of rHuEPO produced by Amgen in 1985 was performed according to the method of Zanetta et al., *J. Chromatogr.* 69, 291 (1972). This analysis shows the presence of fucose as claimed in Claim 9 of EP 411 678. The analysis also reveals the relative molar ratios of hexoses (galactose and mannose) to N-acetylglucosamine to be 1.5 and the ratios of galactose and mannose to N-acetylglucosamine were 0.9 and 0.6, respectively. These values are within the range of experimental and

analytical error to those reported in Claim 10 to EP 411 678 (1.4, 0.9 and 0.5, respectively).

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true.

Date: February 13, 1992

Thomas W. Strickland

Thomas W. Strickland

M 10·11·92

Curriculum Vitae

THOMAS WAYNE STRICKLAND

Date of Birth: 09/14/54

Place of Birth: Pensacola, Florida

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Moorpark, California
(805) 523-8740

Office Address: Amgen Inc., Amgen Center
Thousand Oaks, California 91320
(805) 499-5725 (x3088)

Education:

University of West Florida	B.S., 1976 (Biology)
University of West Florida	B.S., 1976 (Chemistry)
Vanderbilt University	Ph.D., 1981 (Biochemistry)

Ph.D. Thesis:

Contribution of Subunits to Glycoprotein Hormone Function: A Study Utilizing Hybrids

Work Experience:

April 1984 - present
Research Scientist, Protein Chemistry Department, Amgen Inc. Research principally involved in the purification and characterization of urinary and recombinant human erythropoietin

February 1981 - March 1984
Assistant Research Biological Chemist, Department of Biological Chemistry, University of California Los Angeles. Research included studies on biosynthesis and protein folding of glycoprotein hormones; chemical modification, isolation, and receptor cross-linking of glycoprotein hormones

Honors and Offices:

Pensacola Junior College Faculty Scholarship Award (1974)
Foundation Scholarship, University of West Florida (1974-76)
National Science Foundation Undergraduate Research Fellowship (1975)
Monsanto Award for Chemistry (1976)
Graduate Summa Cum Laude, University of West Florida
National Institutes of Health Predoctoral Traineeship (1976-77)
National Science Foundation Predoctoral Fellowship (1977-1980)
Harold Sterling Vanderbilt Graduate Scholarship (1976-1980)

M 10-1-92

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Papers:

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16. The beta-subunit of thyrotropin can be cross-linked to its receptor by disuccinimidyl suberate. P.R. Buckland, T.W. Strickland and B.R. Smith. *Biochemical Society Transactions 13*, 942-943 (1985).

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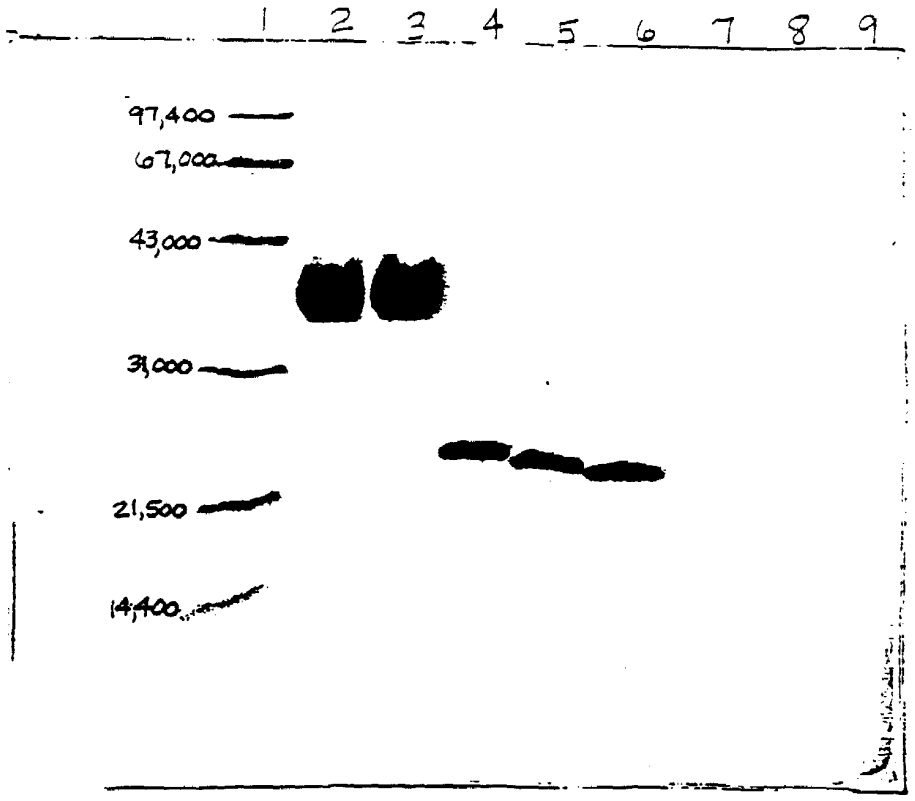
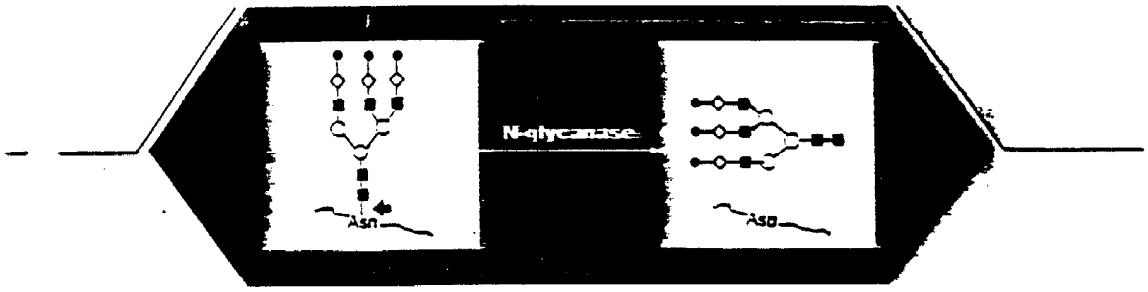


LANE #	SAMPLE #	[illegible]	N-Glycanase	Sialidase	O-Glycanase	37C Incubation
1	Bio-Rad MW Stds	-	-	-	-	-
2	1	+	-	-	-	-
3	2	+	-	-	-	+
4	3	+	+	-	-	+
5	4	+	+	+	-	+
6	5	+	+	+	+	+
7	7	-	+	-	-	+
8	8	-	+	+	-	+
9	9	-	+	+	+	+

NEW FOR GLYCOPROTEIN RESEARCH FROM genzyme

N-glycanase



N-glycanase (Peptide: N-glycosidase F) hydrolyzes the Asn-GlcNAc linkages of glycoproteins to yield:

- Carbohydrate-free intact protein
- Intact oligosaccharides

N-glycanase is:

- Protease-free
- Endo- and exoglycosidase-free

N-glycanase has broad specificity:

GLYCOPEPTIDES:	Genzyme N-glycanase	Endo F	Endo H
Ovalbumin, high Mannose	+	+	-
Ovalbumin, hybrid, with GlcNAc β1→4Man	+	+	-
Ovalbumin, hybrid, without GlcNAc β1→4Man	+	+	-
Ovomucoid Hybrids	+	+	-
Biantennary Complex	+	+	-
Triantennary Complex	+	+	-
Tetraantennary Complex	+	+	-

Personal communication Tarentino and Plummer

N-glycanase allows:

- Accurate molecular weight determination of deglycosylated proteins
- Structural analysis of oligosaccharides
- Analysis of glycoprotein function

genzyme

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Data Sheet

N-GLYCANASE
(Peptide: N-Glycosidase F from
Flavobacterium meningosepticum)

Specific Lot Data
Lot number: 02500 Date of Assay: 2/18/85
Concentration: 260 units/ml by Assay 1

Recommended Storage Conditions:
N-Glycanase is shipped as a 50% glycerol solution containing 2.5 mM EDTA. Storage at -20°C is recommended. Under these conditions the enzyme is stable for at least three months.

Activity Assays:
N-Glycanase activity is quantitated by measuring the release of oligosaccharide from [^{3}H]dansyl-fetuin glycopeptide (1) or from ribonuclease B (2). One unit of enzyme measured by the former assay is the amount of N-glycanase needed to hydrolyze one nanomole of [^{3}H]dansyl-fetuin glycopeptide per minute at 37°C. One unit of enzyme measured by the RNase assay is the amount of N-glycanase required to convert one nanomole of RNase B to RNase A per hour at 37°C.
Conversion Units: One dansyl-fetuin glycopeptide unit is equivalent to 50 RNase units
1. [^{3}H]Dansyl-fetuin glycopeptide assay: 2 µl of [^{3}H] dansyl-fetuin glycopeptide (720 nmol/ml, sp. act. 10 µCi/µmol) and 2 µl of N-glycanase in 0.25M sodium phosphate, pH 8.6, are incubated at 37°C for 30 minutes. The reaction mixture and 2 µl water rinse are spotted on Whatman #3 paper and the chromatogram is developed with butanol/ethanol/water (4:2:3, by volume). The product spot, which migrates near the solvent front, is visualized by UV light and cut from the chromatogram. The product stripe is placed in a scintillation vial with 1.3 ml of 0.5% SDS and heated at 60°C for 5 minutes. Scintillation fluid (10 ml) is added and radioactivity is measured.

RNase assay: A stock solution of RNase B (5 mg/ml) is prepared by boiling the enzyme in 1% SDS for 3 minutes. Twenty-five microgram aliquots of denatured RNase B are incubated with serial dilutions of N-glycanase for one hour at 37°C (recommended range: 1-120 units/ml). The final incubation mixtures contain, in addition, 10 mM β-mercaptoethanol, 0.2M sodium phosphate, pH 8.6, and 0.75% NP-40. The samples are analyzed by SDS-PAGE. The conversion of RNase B to RNase A can be estimated visually or quantitated by densitometry of the stained gel.

Assays for Contaminating Activities
Exoglycosidase Assays
O- and p-nitrophenyl substrates (1 micromole of each) are incubated with N-glycanase (50 units/ml) in a final volume of 0.5 ml for 20 hours at 37°C, pH 5.0. OD_{400} is read after quenching with sodium bicarbonate. The limit of detection of the assay is 1.0×10^{-9} moles of substrate hydrolyzed. Substrates tested:

p-nitrophenyl α-D-galactopyranoside
o-nitrophenyl β-D-galactopyranoside
p-nitrophenyl α-L-fucopyranoside
p-nitrophenyl α-D-mannopyranoside
p-nitrophenyl-N-acetyl-β D-glucosaminide

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Tech-Light Ltd., Hollands Road. Haverhill. Suffolk CB9 8PU. England. Telephone (0440) 702436. Telex 817756

Protease assays

30 nanocuries/ml ^{14}C-bovine serum albumin (New England Nuclear) is incubated with 60 units/ml N-glycanase in a final volume of 35 µl for one hour at 37°C, pH 6.1. No TCA-soluble material could be detected.

Background Information:

Glycanase (Peptide: N-glycosidase F) has been highly purified from cultures of Flavobacterium meningosepticum. The enzyme is distinct from the endo-β-N-acetylglucosaminidase F (Endo F) activity described by Elder and Alexander (1,2). N-Glycanase hydrolyzes N-asparagine-linked oligosaccharides from glycoproteins and glycopeptides to give free oligosaccharide and a peptide/protein containing aspartic acid at the glycosylation site.

The reaction conditions required to affect complete deglycosylation of a glycoprotein with N-glycanase depend on the accessibility and structure of the pendant oligosaccharides. Denatured samples generally require significantly less N-glycanase than do native proteins. All N-linked oligosaccharides examined to date, including bi-, tri-, and tetraantennary complex, hybrid and high mannose chains are cleaved (4). Oligosaccharides attached to a C- or N-terminal amino acid do not appear to be substrates for the enzymes (4). The reaction conditions described below are designed for the deglycosylation of difficult samples. If multiple samples of the same glycoprotein need to be processed, it is recommended that serial dilutions of the enzyme be performed to determine the minimum amount of enzyme required for the reaction. For example, ribonuclease B is completely deglycosylated in one hour at 37°C at a N-glycanase concentration of 1 unit/ml. A representative protocol is given below.

The glycoprotein sample (10 µl, 2.0 mg/ml) is boiled for 3 minutes in the presence of 0.5% SDS + 0.1M β-mercaptoethanol. The sample is diluted with 18 µl of sodium phosphate, pH 8.6, containing 10 mM EDTA and 2 µl of 10% NP-40, 1.8 units (4 µl) of N-glycanase (450 u/ml) is added. The reaction mixture is incubated overnight at 30°C.

References

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GLYCOPROTEIN RESEARCH AND genzyme

N-GLYCANASE

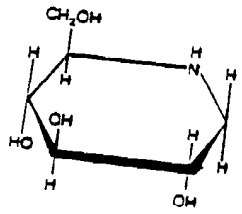
(Peptide: N-glycosidase F)

Specificity: Hydrolysis of Asn-GlcNAc linkages of N-linked glycoproteins

Reference: JBC. 259 : 10700–10704 (1984).

ENDOGLYCOSIDASE H

1-DEOXYNOJIRIMYCIN



Activity: Inhibitor of glycoprotein processing

Reference: JBC. 257 : 14155–14161 (1983).

O-GLYCANASE

(Endo-α-N-acetylgalactosaminidase)

Specificity: Hydrolysis of Gal-GalNAc from Ser (Thr) residues of glycoproteins

Reference: JBC. 252 : 8609–8614 (1977).

genzyme

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For laboratory use only. Not for drug, diagnostic or other use.

genzyme

June 20, 1985.

Dear Researcher,

O-glycanase (endo-α-N-acetylgalactosaminidase) is now available from Genzyme.

The enzyme is purified from cultures of Diplococcus pneumoniae and catalyzes the hydrolysis of the gal β(1-3)galNAc core disaccharide linked to either serine or threonine in glycoproteins. Prior (enzymatic) removal of sialic acid or other saccharides present on this core disaccharide is necessary, as these substituents interfere with O-glycanase activity. GlcNAc β(1-3)galNAc and other structures found linked to serine and threonine are not substrates. Researchers can use O-glycanase as a tool to identify the presence of specific O-linked oligosaccharide chains. Hydrolysis does not require prior denaturation of the glycoprotein of interest, but reaction rates are somewhat faster with glycopeptides than with native glycoproteins.

O-glycanase is available in 50% glycerol solutions shipped to you on dry ice. Prices (excluding shipping and handling) in the continental U.S. are:

Product code: O-ase	25 milliunits	$90.00
	125 milliunits	$250.00

I have listed the following references to provide you with background information on O-glycanase:

Umemoto, J., Bhavanandan, V.P. and Davidson, E.A. J. Biol. Chem., 252, 8609-8614 (1977).

Bhavanandan, V.P., and Davidson, E.A. Biochem. Biophys. Res. Comm., 70, 738-743 (1976).

Lamblin, G., Lhermitte, M., Klein, A., Roussel, P., Van Halbeek, H., and Vliegenthart, J.F.G. Biochem. Soc. Trans., 12, 599-600 (1984).

Please contact me at Genzyme (1-800-332-1042) to obtain additional technical or ordering information.

Sincerely,

Emily J. Levy
Product Manager

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