

UNITED STATES DISTRICT COURT
DISTRICT OF MASSACHUSETTS

AMGEN INC.,)
)
)
 Plaintiff,)
)
 v.)
)
 F. HOFFMANN-LA ROCHE LTD.,)
 a Swiss Company, ROCHE)
 DIAGNOSTICS GmbH, a German)
 Company and HOFFMANN-LA ROCHE)
 INC., a New Jersey Corporation,)
)
)
)
 Defendants.)

Exhibit B

C. A. No.: 05-CV-12237-WGY

PUBLIC VERSION

Exhibit 48

to the Declaration of Cullen N. Pendleton in Support of Amgen’s
Opposition to Roche’s Motion for Summary Judgment that Claim 7 of the ‘349
Patent is Invalid Under 35 § USC 112 and is Not Infringed

RedactedEPOETIN BETA
RO0503821 Starting Material
RO2053859

Roche

Prepared by: HE
Approved by:
Date:**DESCRIPTION OF MANUFACTURING PROCESS AND PROCESS CONTROLS****DETAILED DESCRIPTION OF THE EPOETIN BETA FERMENTATION PROCESS****1. SUMMARY**

The fermentation process of Epoetin beta (EPO) comprises the seed train, the [] production run and the harvest procedure.

The medium for the spinner cultures [] contains methotrexate (MTX), while the medium for the bioreactor cultures [] is free of MTX.

2. OPERATION PROCEDURE OF THE SEED TRAIN**2.1 Spinner Cultivation with MTX**

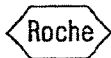
One vial of the working cell bank (WCB) is taken from the vapor phase of the liquid nitrogen storage tank. The cells are transferred into [] spinner flasks and cultivated in a [] buffered medium [] in a humidified [] incubator ($37^{\circ}\text{C} \pm 2^{\circ}\text{C}$). For the composition of the cultivation medium see Table 1 and Table 2. The spinner cultures are microscopically checked for the absence of bacteria and fungi. At each splitting step cell density is counted and the viability is calculated.

After a cultivation phase the cells are transferred to spinner flasks [] [] ($37^{\circ}\text{C} \pm 2^{\circ}\text{C}$). During a [] period, the cells grow from a starting cell density of $0.5 - 3.4 \times 10^5$ cells/mL to a cell density of $2 - 15 \times 10^5$ cells/mL. By splitting, the cell culture is replenished with medium to the starting cell density. Afterwards the cell cultivation is continued.

This procedure is repeated until a culture volume of [] has been obtained. Cultures which meet the given in-process control (IPC) limits for cell age, viability, viable cell density and absence of microbes are available for inoculation of the 10 L inoculum bioreactor (for details see report 3.2.S.2.2 "In-Process Controls During Fermentation").

Spinner flasks are kept as backup cultures up to the maximum cell age in medium with MTX [].

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3. COMPOSITIONS OF THE MEDIA

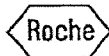
The composition of basal medium DMEM/F12 T005 as shown in Table 1 is used for the spinner cultivation and bioreactor cultivation medium (for details see Table 2).

Table 1 Composition of the Basal Medium DMEM/F12 T005

Components	Concentration [mg/L]	Components	Concentration [mg/L]
L-Alanine	Redacted	myo-Inositol	Redacted
L-Arginine		Nicotinamide	
L-Asparagine/H ₂ O		Pyridoxal/HCl	
L-Aspartic acid		Pyridoxol/HCl	
L-Cysteine/HCl x H ₂ O		Riboflavin	
L-Cystine/2 HCl		Thiamine/HCl	
L-Glutamic acid		Cobalamin	
Glycine			
L-Histidine/HCl x H ₂ O		DL-alpha-Lipoic acid	
L-Isoleucine		Sodium linoleate	
L-Leucine		Sodium pyruvate	
L-Lysine/HCl		Putrescine/2 HCl	
L-Methionine		CaCl ₂	
L-Phenylalanine			
L-Proline			
L-Serine		KCl	
L-Threonine		MgCl ₂	
L-Tryptophan		MgSO ₄	
L-Tyrosine x 2 Na		NaCl	
L-Valine		Na ₂ HPO ₄	
Ca-D(+)pantothenate	NaH ₂ PO ₄		
Choline chloride			
D-Biotin			
Folic acid			
		Redacted	



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The composition of the spinner cultivation medium containing methotrexate (MTX) is shown in Table 2.

Table 2 Composition of the Cultivation Medium for Spinner Cultivation

Medium Components	Concentration [mg/L]
DMEM/F12 T005	11,540
Additionally added compounds	
L-Asparagine monohydrate	[Redacted]
L-Asparaginic acid	
[Redacted]	
L-Serine	
D-Glucose monohydrate	
Hydrocortisone	
Putrescine/2HCl	
[Redacted]	
Sodium bicarbonate	
Sodium chloride	
Sodium selenite pentahydrate	
[Redacted]	
Insulin, recombinant	
Methotrexate	
Phenol red, sodium salt	
Hydrochloric acid	

Redacted