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# Aim and Purpose

This SOP is part of the process description, which contains a plant- and scale-specific description of version 1.2 and version 1.2 OPT of the cell culture process for the production of the humanized, recombinant, monoclonal antibody Product\_Name (bevacizumab, rhuMab VEGF) at the production site Building 95 of F.Hoffmann-La Company AG in Basel (Switzerland).

This document contains acceptance ranges of process parameters that must be met in order to produce a quality-compliant Product\_Name API.

The process description of the cell culture process is divided into process monitoring and IPC, media production, seed fermentation, pre-fermentation and production fermentation with harvesting. The purification process is described in another document.

The umbrella document for documents that are directly related to the manufacturing process of Product\_Name v1.2 and v1.2 OPT at Basel Biotech Manufacturing im Bau 95 (e.g. definition of the recipes used in the process, order templates, definition of tolerance band monitoring, HSE safety instructions, general hygiene regulations, etc.) is [sop024032](https://condorportal.roche.com/condorwp/showContent.action?path=/sop024032&rendition=true).

# Responsibilities

The author and the approving bodies are responsible for the content of this SOP. All employees trained in this SOP are responsible for compliance with this SOP.

# Higher-level and/or co-applicable documents

* [sop024032](https://condorportal.roche.com/condorwp/showContent.action?path=/sop024032&rendition=true): Product\_Name v1.2: Static Part
* [sop022666:](https://condorportal.roche.com/condorwp/showContent.action?path=content/sop022666.pdf) Process Description Building 95: Product\_Name v1.2 - Media Production
* [sop022701:](https://condorportal.roche.com/condorwp/showContent.action?path=/sop022701&rendition=true) Process description Building 95: Product\_Name v1.2 – Seed fermentation
* [sop023038:](https://condorportal.roche.com/condorwp/showContent.action?path=/sop023038&rendition=true) Process Description Building 95: Product\_Name v1.2 – Pre-Fermentation
* [sop022258:](https://condorportal.roche.com/condorwp/showContent.action?path=/sop022258&rendition=true) Process description of construction 95 Product\_Name v1.2: Purification
* [SOP-0121060](https://condorportal.roche.com/condorwp/showContent.action?path=content/SOP-0121060.pdf): Unplanned Events Deviation Management Process
* [SOP-0121059](https://condorportal.roche.com/condorwp/showContent.action?path=content/SOP-0121059.pdf): Planned Events Process
* [SAM-0108905](https://condorportal.roche.com/condorwp/showContent.action?path=content/SAM-0108905.pdf): Bevacizumab v1.2 (OPT), Drug Substance Manufacturing Process Specifications

# Abbreviations and definitions

## Abbreviations

AVA: Product\_Name

CHO cells: Chinese Hamster Ovary Zellen

API: Active Pharmaceutical Ingredient (CCF): Cell Culture Fluid

CIP: Cleaning in place

CPP: Critical Process Parameters: see definitions

DNAF: DNA fluorochromes; Fluorometric detection method for Mycoplasma DNA DO: Dissolved Oxygen

HCCF: Harvested Cell Culture Fluid (geernteter Fermentationsüberstand) ID: Identification number

CPI: In Process Controls

LAL: Limulus amebocyte lysate (determination of endotoxins) LDH: Laktat Dehydrogenase (Lactate dehydrogenase)

LION: Large Volume Ampoule (Ampulla Grossvolumige, 10 ml) Mab: Monoclonal Antibody (Monoklonaler Antikörper)

MCB: Master Zellbank (Master Cell Bank)

MFC: Mass Flow Controller (Massendurchflussregler) MTX: Methotrexat

MVM: Murine minute virus: Parvovirus MZ: Multi-purpose facility

OPT: Optimization

OUR: Oxygen Uptake Rate (Sauerstoffaufnahmerate) PE: Planned Event

PVC: Packed Cell Volume = centrifuged cell volume PM: Process monitoring

PP3: Proteosis Peptone 3 = Proteosis Peptone 3 Solution 20% RPM: Rotation per minute SIP: Sanitization in place

SSF: South San Francisco

TR: 20L Seed-Train-Bioreactor = Seedfermenter VEGF: Vascular Endothelial Growth Factor

RRP: Unplanned Event

WCB: Arbeitszellbank (Working Cell Bank)

WFI: Water for injection (Wasser für Injektionszwecke)

324K: SV40 (simian virus 40)-transformed human newborn kidney: Zelllinie für Parvo- Virus Test

## Definitions

**20L Seed Fermenter:** 20L Seed-Train-Bioreactor (STB, designation according to Genentech)

**Seed fermentation:** Seed Train (name according to Genentech)

**Pre-fermenter/pre-fermentation:**  Inoculum Bioreactor/Inoculum Train (designation according to Genentech)

Production Bioreactor/Production Train (Genentech designation)

**Process monitoring (PM):**Analytics that are carried out during the production process

**In-Process Controls (IPC):** Analysis of samples drawn during the manufacturing process, which is used to monitor and, if necessary, adjust the processes with regard to compliance with specifications and is carried out by external employees.

**Setpoint: Setpoint of the process parameter**

**Setpoint Range**: The work area in which the process is conducted. The setpoint range is within the acceptance range.

**Acceptable Range:** Defined range of values of the process parameter for which compliance with the final product quality specifications has been proven (Proven Acceptable Range).

**Alert Limit:** *Limits* of the setpoint range to provide early warning of possible deviations from normal operating conditions. Exceedances do not necessarily require corrective action, but do give rise to appropriate consideration of potential problems. Warning limits are always tighter than action limits.

**Action Limit:**  *Acceptance Limit of* the process parameter or narrower value. Any violation of the action limit that occurs during the process is [to be handled](https://condorportal.roche.com/condorwp/showContent.action?path=content/SOP-0121060.pdf) in accordance with SOP-0121060.

**Medium/Media:** Umbrella term for cell culture media, stick and addition solutions

**CPP: Critical process** parameter: Quality-relevant process parameters that are controlled within predetermined criteria, as they have an influence on the target variables (quality characteristic of the product and its yield).

**Product\_Name v1.2 OPT:** unlike Product\_Name v1.2, media without gentamycin are used. In addition, the fumigation strategy differs (max. air fumigation, as well as the dissolved oxygen). An addition solution is used, as well as a smaller amount of batch feed.

**HCCF Storage Start/End:** The start of HCCF storage is defined as the date and time when the HCCF has been completely transferred to the harvesting tank. The end of HCCF storage is defined as the date and time at which the transfer of the HCCF to the next step (protein A chromatography) begins.

**Split Ratio:** Dilution factor for the next stage of cell culture. Calculation of the ratio of the total quantity to the inoke quantity.

**Transfer Cell Density:** The cell density of the transmitter bioreactor immediately prior to transfer.

**Inoculation cell density:** The cell density of the receiver bioreactor immediately after transfer, or after inoculation.

# Scope

The aim of the production fermentation process is to produce bevacizumab. During the harvesting process, bevacizumab-containing cell culture fluid is separated from cells and cell debris.

# Overview of the Cell Culture Process

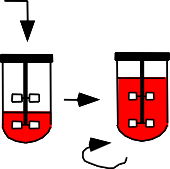
This section provides an overview of the cell culture process. The goal of the cell culture process is to produce harvested cell culture fluid (HCCF) on a production scale. Figure 1 shows the sequence of the individual steps of the cell culture process up to the production fermenter stage.

##### Seedfermentation

##### Pre-fermentation

##### Production fermentation



10 mL

Ampoule

N-3 N-2 N-1

Harvest



of the cell culture supernatant

~ 5L 20L



Selektives Medium

Multiple passages in non-selective medium

Non-selective production medium

(20L) (80L, 400L, 2000L) (12'500L)

##### Figure 1: Product\_Name v1.2 and v1.2 OPT cell culture process

At the start time, two large-volume ampoules (10ml LVA) of the working cell bank (WCB) are thawed and transferred to a 20L seed fermenter. The culture is expanded to the working volume of the 20L seed fermenter and then obtained by means of subcultivation as a seed supplier for pre-fermentation or further 20L seed fermenters. The 20L seed cultures are run with selective medium (contains methotrexate).

The 80L pre-fermenter (N-3) is inoculated with seed material from a 20L seed fermenter and expanded to the further pre-fermentation stages N-2 (400L) and N-1 (2000L). As a rule, the cultivation of the cells in the pre-fermenters takes a total of 9-10 days. The 12,500L production batch inoculated with the pre-culture is run with feeding (bolus feed) and lasts approx. 14 days. This is followed by harvesting, in which the cell mass is separated from the cell culture fluid by centrifugation.

##### Table 1: Process parameters process version 1.2 and v1.2 OPT

|  |  |
| --- | --- |
| **Parameter** | **Value or description** |
| Expression System | CHO DP12 |
| Cell line | aVEGF G7 / MCB #2055 |
| Antibody isoelectric point | 8.2 |
| Absorbance coefficient (A280 nm) of the antibody | 1.7 mL / (mg x cm) |
| Molecular weight of the antibody | 149'232 Dalton |
| Type of cell culture process | Fed-batch suspension culture |

1. **Production fermentation**

**(Material numbers: 10078194, 10185709)**

## Process Overview

The third stage of the Product\_Name manufacturing process is production fermentation (N) and is run in a 12,500L fermenter. During this production phase, the cells produce the antibody bevacizumab. This fermenter is fed with a fixed amount of medium and inoculated with inoculum from a 2000L fermenter. The control and monitoring of the fermentation parameters is carried out according to the information in Table 5 for Product\_Name v1.2 and Table 6 Product\_Name v1.2 OPT. The monitoring of the culture as well as the sampling are carried out according to the sampling plan ([spt017981](https://condorportal.roche.com/condorwp/showContent.action?path=/spt017981&rendition=true)) over the entire duration of the production culture.

In production fermentation, two oblique blade stirrers are used to ensure sufficiently good mixing over the entire height of the fermenter.

The cells are cultured in a non-selective medium containing peptone PP3. For Product\_Name v1.2 OPT, an additional solution is added to the media. After approximately 64 hours, the temperature is reduced from 37°C to 33°C. After approximately 72 hours or sooner, if the glucose concentration drops below 3 g/L, a concentrated batch feed solution is added to replace consumed nutrients. This increases the viability of the cells over the duration of cultivation, as well as productivity. In addition, the addition of a 50% glucose solution (equivalent to the addition of 6 g of glucose per L of cell culture) is required if the glucose concentration in the cell culture falls below 3 g/L to avoid glucose deficiency. Typically, one to two of these glucose additions are needed per production fermentation. The duration of production fermentation is determined by the following three parameters:

* The duration of production fermentation must be between 240 and 384 hours.
* Production fermentation must be completed before or when the maximum cell age is reached. The maximum cell age is WCB-specific and is counted from the thawing date.
* The cultivation period in non-selective medium (calculated from the start of the N-3 culture to harvest) must not exceed 28 days.

A sample for the test for the presence of rodent parvovirus (PCR test) must be taken 2-6 days before harvest. At the end of the production fermentation, samples are taken and examined for bioload. Furthermore, a test for rodent parvoviruses (PCR test), for leptospira (PCR test), a mycoplasma test, and an *in vitro* virus screening test are performed. Harvesting is carried out in order to obtain and purify the product-containing supernatant of the cell culture fluid.

##### Table 2: Summary: Cell Culture Parameters in Production Fermentation

|  |  |
| --- | --- |
| **Cell Culture Operating Parameters** | **Value** |
| Maximum cultivation time in non-selective medium | 28 days |
| Culture medium  **Product\_Name v1.2:** Genisys Nr. 10076320 | Production medium 12'500L |
| **Avast v1.2 OPT:** Genisys Nr. 10185710 |
| Typical Medium Batch Quantity (kg) | 8260 |
| Typical Amount of Addition Solution Production Medium Genisys No. 10185716 (kg) | 41 |
| Typical Inoculum Amounts (N-1) (kg) | 2340 |
| Typical Batch Feed Quantity (kg),  **Product\_Name v1.2:** Genisys Nr. 10078763 | 1670 |
| **Product\_Name v1.2 OPT:** Genisys Nr. 10198547 | 1397 |
| Typical amount of 50% glucose solution (Genisys No. 10076290) (kg) | 169 |
| Typical number of glucose additions | 1-2 |
| Vorgelegte Menge sodium carbonat (Genisys No.  10081866) (kg) | 380 |
| Typical final crop quantity (kg)1 | 12700 |
| 1 Changes in quantity due to anti-foam additions and sampling are negligible and have been disregarded. | |

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## Plant Specifications

##### Table 3: 12,500L Production Fermenter Equipment Specifications

|  |  |
| --- | --- |
| **Parameter** | **Value** |
| MZ – Numbers | 241, 242, 243, 244, 245, 246 |
| Upper Stirrer: Type | Oblique blade stirrer |
| Upper Stirrer: Diameter | 66 cm |
| Bottom Stirrer: Type | Oblique blade stirrer |
| Bottom Stirrer: Diameter | 66 cm |
| Fumigator: Type | L-tube, open (inner diameter: 18.1 mm) |

## Process-relevant control strategies and parameters

Table 4 describes the control strategies used.

##### Table 4: The Rules Strategy

|  |  |
| --- | --- |
| **Regulator** | **Strategies** |
| Temperature | Automatic control, heated double jacket |
| ph | Automatic control. Dosing of sodium carbonate solution via caustic storage container (gravity) and CO2 dosing via mass flow controller  with 0.03 pH units dead tape |
| Dissolved oxygen | Automatic control, mixed gas gassing strategy with air and O2, the maximum total gassing volume flow (air and oxygen) is fixed |
| Stirrer | Automatic control |
| Overpressure | Automatic control, pressure relief valve in the exhaust pipe |

Table 5 describes the parameters used for the production fermentation of Product\_Name v1.2.

##### Table 5: 12'500L N Production Fermenter Parameters of Product\_Name v1.2

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Parameter** | **CPP** | **Setpoint** | **Setpoint range** | **Acceptance area** | **Registered area** |
| Medium Batch Menge (kg)1 | No | 8260 | 7847-8673 | 7434-9086 | Unspecified |
| Inoculum Menge (kg) | No | 2340 | 2223-2457 | N/A | Unspecified |
| Batch Feed Addition Quantity (kg) 1 | No | 1670 | 1586-1753 | 696-2783 | 0.063-0.250 L/L  Culture (equivalent to 0.066-0.263 kg/kg culture) |
| Total amount of culture (kg) | No | 12700 | N/A | N/A | Unspecified |
| Split Ratio | No | 4.5 | N/A | N/A | Unspecified |
| Initial temperature (ºC)9 | And | 37 | 36.5-37.5 | 36-38,  29-36/38-39  for ≤ 2 hours | 36-38,  29-36/38-39  for ≤ 2 hours |
| Temperature reduction time (hours) | No | 64 | 60-68 | 40-88 | 40-88 |

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|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Parameter** | **CPP** | **Setpoint** | **Setpoint range** | **Acceptance area** | **Registered area** |
| Temperature after lowering (ºC)9 | And | 33 | 32.5-33.5 | 32-34,  29-32/34-39  for ≤ 2 hours | 32-34,  29-32/34-39  for ≤ 2 hours |
| pH9 | And | 7.15 | 7.0-7.3 | 6.85-7.35,  6.6-6.85/7.35-7.7  for ≤ 12 hours | 6.85-7.35,  6.6-6.85/7.35-7.7 for  ≤ 12 hours |
| Dissolved oxygen (%)2,  9 | No | 30 | 15-80 | 15-80,  5-15/80-120  for ≤ 16 hours | 15-80,  5-15/80-120  for ≤ 16 hours |
| Overpressure (mbar) | No | 150 | N/A | N/A | N/A |
| Air Headspace Fumigation(L/min) | No | 250 | N/A | N/A | Unspecified |
| Min Air Fumigation (L/min)10 | No | 11 | N/A | N/A | Unspecified |
| Max Air Fumigation(L/min) | No | 250 | N/A | N/A | Unspecified |
| Max O2 gassing (L/min)3 | No | 93 | N/A | N/A | Unspecified |
| Max CO2-Begasung (L/min) | No | 75 | N/A | N/A | Unspecified |
| Stirrer speed (rpm)4 | No | 65 | > 45 | N/A | Unspecified |
| Stirrer-specific power distribution  (cm2/s3)5 | No | 383 | N/A | N/A | Unspecified |
| Initial anti-foam addition6 | No | 2.5 kg 3% simethicone solution  (dead volume)  Quantity: 0.27 kg) | N/A | N/A | Unspecified |
| Anti-foam application6 before batch feed | No | 0.5 kg 3% simethicone solution | N/A | N/A | Unspecified |
| Addition of sodium carbonate solution | No | As needed (controlled by pH regulator) | N/A | N/A | Unspecified |
| Batch Feed Addition Time | No | 72 hours or sooner if < 3g/L glucose | 68-76 hrs | 52-96 hrs | 52-96 hrs |
| Amount of 50% glucose solution (kg)7 | No | 169 | 160-178 | N/A | Unspecified |
| Glucose Addition Criterion8 | No | If [glucose] <  3.0 g/L according to the previous  Glucose Addition | N/A | N/A | If < 3 g/L glucose |
| Inoculation Cell Density (%PCV) | Yes | 0.30 | 0.27-0.33 | 0.12-0.43 | 0.12-0.43 |

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Parameter** | **CPP** | **Setpoint** | **Setpoint range** | **Acceptance area** | **Registered area** |
| Cultivation time (hours) | No | 336 | 324-348 | 240-384 | 240-384 |
| 1 The osmolality values and the shelf life of the medium and batch feed can be found in the [sop022666:](https://condorportal.roche.com/condorwp/showContent.action?path=content/sop022666.pdf) "Process Description Construction 95: Product\_Name v1.2 – Media Production".  2 The DO is above the upper limit of 80% shortly after inoculation, and once it has dropped below 80%, the value must be in the setpoint range. Short-term falls below the DO concentration of max. 96 seconds (values <5%) are not to be regarded as UPEs. Such shortfalls are due to the inertia of the pO2 regulator.  3 The maximum O2 flow is based on the safety limits of Building 95. For process engineering reasons, however, there is no limit to the maximum O2 flow.  4 The value given is based on calculation in order to achieve a constant energy input.  5 A power number of 1.5 is used for the lower stirrer and 1.5 for the upper stirrer.  6 The recipe-controlled anti-foam application takes place after the reactor has been filled with medium and before the batch feed. During the rest of the cultivation period, the addition is controlled by the foam probe.  7 Amount corresponds to 6 g glucose/L cell culture fluid.  8 The addition of glucose can be omitted at a concentration < 3 g/L if the harvest takes place within the next 24 hours or after consultation with Process Engineering.  9 The permitted deviations during the process relate to the respective event and are considered individually. If the deviations occur repeatedly, an assessment must be made.  The setpoint, acceptance and registered ranges shown are relevant for the online pH value and are monitored in the MES. The pH value is regulated by the value measured by the online pH probes. The measurement of the offline pH value is used for process monitoring and the detection of probe drift and a comparison of online and offline pH values is carried out, which, depending on the difference value and time in the process, can lead to an adjustment of the online pH probes.  10 No minimum flow is required. However, due to the precision of the mass flow controller, the flow rates between 0 L/min and the specified minimum flow rate are not accurate. General: the short-term departure from the acceptance areas is not considered a UPE if this is the result of a transfer (e.g. during inoculation) or when adjusting parameters to the inertia of the  regulator. | | | | | |

Table 6 describes the parameters used for the production fermentation of Product\_Name 1.2 OPT.

##### Table 6: 12'500L N Production Fermenter Parameters of Product\_Name v1.2 OPT

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Parameter** | **CPP** | **Setpoint** | **Setpoint range** | **Acceptance area** | **Registered area** |
| Medium Batch Menge (kg)1 | No | 8260 | 7847-8673 | 7434-9086 | Unspecified |
| Addition solution (kg) | Yes | 42.1 kg (41 kg  Addition solution + 1.1 kg dead volume) | 38 kg – 46.2 kg  (36.9 – 45.1  Encore solution +  1.1 kg Totvolumen) | 36.6 kg – 68.5 kg  (35.5 kg – 67.4 kg  Encore solution +  1.1 kg Totvolumen) | 36.6 kg – 68.5 kg  (35.5 kg – 67.4 kg  Encore solution +  1.1 kg Totvolumen) |
| Inoculum Menge (kg) | No | 2340 | 2223-2457 | N/A | Unspecified |
| Batch Feed Addition Quantity (kg) 1 | No | 1397 | 1327-1467 | 704-2793 | 0.063-0.250 L/L  Culture (equivalent to 0.066-0.263 kg/kg culture) |
| Total amount of culture (kg) | No | 12700 | N/A | N/A | Unspecified |
| Split Ratio | No | 4.5 | N/A | N/A | Unspecified |
| Initial temperature (ºC)9 | And | 37 | 36.5-37.5 | 36-38,  29-36/38-39  for ≤ 2 hours | 36-38,  29-36/38-39  for ≤ 2 hours |
| Temperature reduction time (hours) | No | 64 | 60-68 | 40-88 | 40-88 |
| Temperature after lowering (ºC)9 | And | 33 | 32.5-33.5 | 32-34,  29-32/34-39  for ≤ 2 hours | 32-34,  29-32/34-39  for ≤ 2 hours |
| pH9 | And | 7.15 | 7.0-7.3 | 6.85-7.35,  6.6-6.85/7.35-7.7  for ≤ 12 hours | 6.85-7.35,  6.6-6.85/7.35-7.7  for ≤ 12 hours |
| Dissolved oxygen (%)2,  9 | No | 60 | 15-80 | 15-80,  5-15/80-120  for ≤ 16 hours | 15-80,  5-15/80-120  for ≤ 16 hours |
| Overpressure (mbar) | No | 150 | N/A | N/A | N/A |
| Air Headspace Fumigation(L/min) | No | 250 | N/A | N/A | Unspecified |
| Min Air Fumigation (L/min)10 | No | 11 | N/A | N/A | Unspecified |
| Max Air Fumigation(L/min) | No | 250 | N/A | N/A | Unspecified |
| Max O2 gassing (L/min)3 | No | 93 | N/A | N/A | Unspecified |
| Max CO2-Begasung (L/min) | No | 75 | N/A | N/A | Unspecified |
| Stirrer speed (rpm)4 | No | 65 | > 45 | N/A | Unspecified |

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Parameter** | **CPP** | **Setpoint** | **Setpoint range** | **Acceptance area** | **Registered area** |
| Stirrer-specific power distribution (cm2/s3)5 | No | 383 | N/A | N/A | Unspecified |
| Initial anti-foam addition6 | No | 2.5 kg 3% simethicone solution  (dead volume)  Quantity: 0.27 kg) | N/A | N/A | Unspecified |
| Anti-foam application6 before batch feed | No | 0.5 kg 3% simethicone solution | N/A | N/A | Unspecified |
| Addition of sodium carbonate solution | No | As needed (controlled by pH regulator) | N/A | N/A | Unspecified |
| Batch Feed Addition Time | No | 72 hours or sooner if < 3g/L glucose | 68-76 hrs | 52-96 hrs | 52-96 hrs |
| Amount of 50% glucose solution (kg)7 | No | 169 | 160-178 | N/A | Unspecified |
| Glucose Addition Criterion8 | No | If [glucose] <  3.0 g/L according to the previous  Glucose Addition | N/A | N/A | If < 3 g/L glucose |
| Inoculation Cell Density (%PCV) | Yes | 0.30 | 0.27-0.33 | 0.12-0.43 | 0.12-0.43 |
| Cultivation time (hours) | No | 336 | 324-348 | 240-384 | 240-384 |

1 The osmolality values and the shelf life of the medium and batch feed can be found in the [sop022666:](https://condorportal.roche.com/condorwp/showContent.action?path=content/sop022666.pdf) "Process Description Construction 95: Product\_Name v1.2 – Media Production".

2 The DO is above the upper limit of 80% shortly after inoculation, and once it has dropped below 80%, the value must be in the setpoint range. Short-term falls below the DO concentration of max. 96 seconds (values <5%) are not to be regarded as UPEs. Such shortfalls are due to the inertia of the pO2 regulator.

3 The maximum O2 flow is based on the safety limits of Building 95. For process engineering reasons, however, there is no limit to the maximum O2 flow.

4 The value given is based on calculation in order to achieve a constant energy input. 5 A power number of 1.5 is used for the lower stirrer and 1.5 for the upper stirrer.

6 The recipe-controlled anti-foam application takes place after the reactor has been filled with medium and before the batch feed. During the rest of the cultivation period, the addition is controlled by the foam probe.

7 Amount corresponds to 6 g glucose/L cell culture fluid.

8 The addition of glucose can be omitted at a concentration < 3 g/L if the harvest takes place within the next 24 hours or after consultation with Process Engineering.

9 The permitted deviations during the process relate to the respective event and are considered individually. If the deviations occur repeatedly, an assessment must be made.

10 No minimum flow is required. However, due to the precision of the mass flow controller, the flow rates between 0 L/min and the specified minimum flow rate are not accurate.

General: leaving the acceptance areas for a short time is not considered a UPE if this is the

The result of a transfer (e.g. during inoculation) or when setting parameters is due to the inertia of the controllers.

## Monitoring of fermentation

### Fermenter monitoring (online data)

The fermenters are regularly monitored. The online data acquisition includes pH, dissolved oxygen and temperature (both control probe and control probe data are recorded). The gassing rates for all flow meters of the sparger and headspace gassing, the remaining quantities of sodium carbonate solution and antifoam, the pressure of the fermenter and the speed of the stirrer are also recorded.

### Fermenter samples (offline measurements)

Samples are taken from the fermenters at regular intervals in order to carry out various analyses (according to [spt017981](https://condorportal.roche.com/condorwp/showContent.action?path=content/spt017981.pdf)). The results of these analyses serve as the basis for various decisions during the process.

A sample must be taken and analysed from a production fermenter at least after inoculation, on day 3 after completion of batch feed addition and daily from day 7 onwards. The interval between each sampling from day 7 onwards must usually be approximately 24 hours, but must never be longer than 30 hours.

The samples are taken under aseptic conditions. For samples for which a separate flow is drawn (e.g. Gore sampling system), this flow can be disposed of, together with the sample, after receiving the valid results of the process monitoring measurements.

An exception to this is the sample "AVA-vorChrom" or "AVO\_vorChrom", for which the flow [must be stored at ≤-60°C](https://condorportal.roche.com/condorwp/showContent.action?path=content/spt017981.pdf) as a retention sample in accordance with spt017981.

When the Novaseptum sampling system is used, the pre-flow pattern is omitted. The sample is aseptically drawn at the plant. In this case, both MES labels are glued to the Nova bag. According to [spt017981](https://condorportal.roche.com/condorwp/showContent.action?path=content/spt017981.pdf) , this can be disposed of after receipt of all valid results of the process monitoring measurements. There is no mandatory storage.

#### Process monitoring

For offline measurement of pH and glucose concentration, a cobas\_b221 or equivalent device is used. Based on this offline pH measurement, the pH probes of the fermenter can be adjusted. The procedure for pH adjustment is defined as follows: for the media sample before inoculation as well as the cell culture sample directly after inoculation, the control probe and the control probe are adjusted to the pH value measured offline. For all samples thereafter, the control probe is adjusted by 0.05 pH units if the online pH (measured by the control probe) deviates by more than 0.1 pH units from the measured offline pH. The control probe is adjusted to the pH value of the control probe. A detailed procedure with regard to pH adjustments and the procedure in the event of a differential alarm is described in [spt017322](https://condorportal.roche.com/condorwp/showContent.action?path=content/spt017322.pdf) .

Depending on the result of the glucose determination, glucose solution or batch feed are added. Batch feed is added 72 hours after inoculation of the production fermenter or sooner if the glucose concentration is < 3 g/L. Glucose is added after batch feed if the glucose concentration is < 3 g/L.

The addition of glucose can be omitted at a concentration < 3 g/L if the harvest takes place within the next 24 hours or after consultation with Process Engineering.

At a glucose concentration of 3.0 - 3.4 g/L, additional sampling is carried out within the next 12 to 18 hours.

The biomass of the digester is monitored by measuring the so-called "Packed Cell Volume" (PCV). To determine the PCV value, suitable glass tubes (6.5 or 10 ml) and a centrifuge (830g during 10 min) are used.

#### Key data of comparability in cell culture

The following cell culture data are used for comparability:

* + - * + **Final integrated viable Packed Cell Volume (ivPCV) (mL-day/L)** calculated from viability and PCV,
        + **Viability (%)** of cells before harvest (see chapter 7.4.2.3).

##### Finaler Titer CCF

#### Optional Analyses

In addition to the analyses performed for process monitoring, the parameters pCO2 saturation and lactate concentration are determined to monitor the culture. The osmolality of the culture is measured by means of an osmometer that works according to the principle of freezing point reduction ([sop022057](https://condorportal.roche.com/condorwp/showContent.action?path=content/sop022057.pdf)). Live cell density and viability are determined by means of a color exclusion method (trypan blue). An automated cell counting device (e.g. Cedex HiRes or equivalent) is usually used for this purpose. In addition, manual cell counting with a hemocytometer can also be used to determine live cell density ([sop018098](https://condorportal.roche.com/condorwp/showContent.action?path=content/sop018098.pdf)).

The determination of pO2 saturation as well as the concentrations of sodium, potassium, ammonium, glutamate and glutamine can be carried out additionally if necessary. Other measurements that correlate with cell mass and/or cell viability, such as optical density, oxygen uptake rate (OUR) or lactate dehydrogenase activity (LDH activity), can also be performed.

The analysis results of these optional measurements are not used for process decisions in the main fermentation, but only serve as information for extended analysis in the event of troubleshooting.

## 12'500L Production Fermenter: Process Flow

1. Fermenter preparation: Prepare the purified fermenter technically, including equipping it with filters and equipping it with 2 calibrated pH and 2 calibrated pO2 probes. Perform the fermenter leak test.
2. Sanitization: Sanitize the fermenter and its gas filter, medium, batch feed and caustic template and then superimpose pressure by means of headspace gassing.

3 Feeding: Connect the container with the antifoam solution to the fermenter and sanitize its connector. Transfer the required quantities of lye to the template. Transfer the required amount of medium to the fermenter. Activate the stirrer and set the temperature set to 37°C.

1. Antifoam Addition: Transfer the required amount of antifoam into the fermenter.
2. pH probe adjustment: Activate pH control. If the difference between the pH values of the pH control probe and the pH control probe is too large, a delta alarm is triggered. If necessary, the pH value of the pH control probe is adjusted to the pH value of the pH control probe.
3. pO2 probe adjustment: When the medium is saturated with oxygen at constant air gassing, the two pO2 probes are adjusted to 95% air saturation. Then activate the pO2 control.
4. Hold medium: During this phase, the pH, temperature and pO2 controls are activated, but the caustic addition remains inactive or becomes inactivated. Manual adjustment of the pH values of the pH control probe and the pH control probe. If necessary, the pH value of the pH control probe is adjusted to the pH value of the pH control probe.
5. Product\_Name v1.2 OPT Process: Add the required amount of solution to the production medium. It must be noted that the addition solution must be added to the medium immediately before inoculation.
6. pH probe adjustment: Take a sample and measure its pH immediately before inoculation. Adjusting the two pH probes of the fermenter.
7. Inoculation Sanitization of the transfer line between the 2000L and the 12,500L fermenter. Inoculation of the fermenter with the complete inoculation material from the 2000L fermenter. Trial purchase within 60 minutes after inoculation to determine the offline data and adjust Both pH Probes of Fermenters.
8. Fermentation phase: During the fermentation phase, a sample must be taken at least after inoculation (see point 10), on day 3 after completion of the batch feed addition and from day 7 every 24 hours (there must not be more than 30 hours between 2 samplings) and proceed according to [spt017981](https://condorportal.roche.com/condorwp/showContent.action?path=content/spt017981.pdf) . The fermentation parameters are recorded via the data acquisition.

64 hours after the start of production fermentation, the temperature must be lowered from 37 to 33°C. Approximately 72 hours after the start of production fermentation, or sooner if the glucose concentration drops below 3 g/L, add the Batch Feed Medium. The batch feed medium is stirred in the batch feed media template (setpoint: 300 rpm, see also val002652). Glucose addition(s) are to be carried out as required.

## Filters used

Filter information for liquids can be [found in](https://phoenixportal.roche.com/glossary/glossary/sop022666)  sop022666.

##### Hydrophobic gas filters

In principle, all gas filters qualified for the respective installation position (supply air, exhaust air and sparger filters) may be used at the respective location. Filters that are not directly stored in the recipe can be used manually in the running subbatch. The resulting exceptions in the MES can be commented on with reference to [sop023326](https://phoenixportal.roche.com/glossary/glossary/sop023326) . In contrast to liquid filters, gas filters are not product-specific and are therefore not explicitly listed in the process descriptions. The filters that are qualified and approved for the respective locations are stored in the master data in the MES and can be viewed via the corresponding MES reports.

# Harvest

Harvesting is the final step of fermentation and is used to separate CHO cells and their cell debris from the cell culture fluid (CCF). The product is included in the supernatant. Furthermore, the harvest serves to clarify this supernatant in order to obtain the harvested cell culture fluid (HCCF). The separation of the cells and cell debris is carried out with a separator. The supernatant is then clarified by a combination of depth filters and membrane filters (double layer 0.45m/0.2m).

## Summary of the operation

The separator is continuously fed with cell culture fluid. The solids are separated in the separator drum and discharged periodically (ejection). An interval volume is calculated on the basis of the PCV, after which the ejection takes place periodically. The solids are fed to the Water Wastewater Industry, Infected (WAI-Inf), where they are treated and disposed of accordingly. The cell culture fluid from the separator (centrate) is then clarified through a series of depth and membrane filters and pooled as clarified HCCF. Water for injection (WFI) is used to flush the centrifuge. The media collector enables a shock-free change between product from the production fermenter and WFI. In preparation for the filter change, the filters of the harvesting line are emptied into the WAI by means of air pressure after WFI rinsing.

The harvested cell culture fluid is stored for further processing (see specification Table 8).

When transferring the HCCF from USP to DSP, transfer stops may occur due to the control behavior of the pump. Continuous pump stops, which last 10 minutes, create a fault "STOE\_PROZ\_STROM" and automatic exception. The interruption must be evaluated in accordance with [SOP-0121060](https://condorportal.roche.com/condorwp/showContent.action?path=content/SOP-0121060.pdf) as part of a UPE. See risk assessment CR TW#[1606522](https://trackwise-boyer.roche.com/TeamAccess/Gateway.html?1606522).

##### Table 8: Process Parameters of Harvesting and HCCF Storage

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Parameter** | | | | **Setpoint or description** | **Acceptance Area** | **Registered area** |
| Separator Type | | | | Alfa-Laval BTAX 215H-31CEP  (hermetic) | N/A | N/A |
| Separator speed (rpm) f | | | | 5500 (equals 9880 x g) | 5000-6000 | 5000-6000 |
| Rinse (WFI, before product) | Zuflussrated | | | 80-100 L/min  (4800-6000 L/h) | ≥ 80 L/min  (≥ 4800 L/h) | N/A |
| Volu- men (L)c | | 1.  Step | 1640 | ≥ 1600 | N/A |
| 2.  Step | 2200 | ≥ 2150 |
| 3.  Step | ≥ 253 | N/A | N/A |
| CCF – Inlet pressure (fermenter overpressure at harvest) (mbar)g | | | | 200-1000 | 200-1000 | N/A |
| Intervall-Volumen (L) | | | | The interval volume is variable and is calculated based on the PCV:  *SolidsVolume*(8L)*x100*  *PCV*[%] | N/A | N/A |
| Harvest (CCF) Inflow Rate | | | | 60 L/min  (3600 L/Std) | 40-80 L/min  (2400-4800 L/Std) | 40-80 L/min (2400-  4800 L/Std) |
| Differential Pressure Inlet Filter Harvest Tank (mbar) | | | | N/A | ≤ 2068 | ≤ 2068 |
| After Harvest: Product Removal | | Solution | | WFI | N/A | N/A |
| Volume (L) | | ≥ 900 | ≥ 800 | N/A |
| Purification (CIP) Inflow Rate | | | | 50-80 L/min  (3000-4800 L/Std) | N/A | N/A |
| Jacket Cooling Separator | | | | Separator cooling with WFI 20°C | N/A | N/A |
| Required water pressure (bar) | | | | 2-4 | N/A | N/A |
| Operation temperature (except HCCF harvesting tank) (ºC) | | | | 15-38 | N/A | N/A |
| Operation Temperature HCCF Harvesting Tank (ºC) | | | | 12-18 | N/A | N/A |
| Minimum HCCF product titer (g/L) | | | | ≥0.65 | ≥0.65 | ≥0.45 |
| Product yield (average)a | | | | 92% (non-PCV adjusted)  95% (PCV-angepasst) | N/A | N/A |
| HCCF-Poolvolume (L) | | | | 12390 (11150-13650) | N/A | N/A |
| Rotational speed harvesting tank (rpm) b | | | | 50 | N/A | N/A |

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|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Parameter** | | **Setpoint or description** | **Acceptance Area** | **Registered area** |
| Ava 1.2 HCCF Pool Storage Time (days)h,i | at 15 ± 3°C | ≤ 5 | ≤ 5 | ≤ 5 |
| Ava 1.2 opt HCCF Pool Storage Time (days)h,i | at 15 ± 3°C | ≤ 4 | ≤ 4 | ≤ 5 |
| a Average crop yields from 16 Product\_Name v1.2 approaches from Genentech.  b The stirrer of the harvest tank can also run intermittently during storage.  c The harvest filters are connected in series. Furthermore, there is a bypass valve between each filter module. The flushing of the first stage only goes through the first filter and bypasses the others. After rinsing at least 800 L, the second stage rinses the first and second filters at a minimum of 2000 L, bypassing the third filter. In the third stage, all three harvest filters are flushed with at least 253 L.  d Due to the requirement time for WFI, there may be a delay/start-up variation during stage 1 flushing. Due to pressure differences between the filter stages, the WFI inflow rate can fluctuate briefly between flushing stage 1 and 2. Both are typical of the process and are to be evaluated as uncritical. These delays generate an automatic exception, but do not need to be evaluated according to [SOP-0121060](https://condorportal.roche.com/condorwp/showContent.action?path=content/SOP-0121060.pdf) as part of a UPE.  e, f During centrifugation, ejections take place. Parameter fluctuations that take place during these ejections are not to be evaluated in the evaluation.  g The acceptance range for the fermenter overpressure is only to be evaluated for the transfer time from the fermenter.  h According to Genentech's VP05-36 validation, the storage of the HCCF pool at 25 ± 2°C for ≤ 2 days is also covered for product stability and is practiced at other Product\_Name v1.2 producing sites. The durability of the HCCF pool in terms of microbiological integrity has been proven according to val006000 for 5 ± 3°C ≤10 days and for 15 ± 3°C ≤ 5 days.  However, due to a requirement of the Chinese regulatory authority as part of the approval of the MIT dossier submitted in Q2 2015, a combination of storage temperatures is to be dispensed with, only 15°C ± 3°C will be used as storage temperature.  i HCCF Storage Start/End: The start of HCCF storage is defined as the date and time at which the HCCF has been completely transferred to the harvesting tank. The end of HCCF storage is defined as the date and time at which the transfer of the HCCF to the next step (protein A chromatography) begins.  General: The above parameters are not critical in terms of product quality; however, if the minimum HCCF titer is not reached, the purification of the batch cannot be carried out for technical reasons. | | | | |

##### able 9: Filter Configuration

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Filter- Position | Description | Genisys-Nr. | Manufacturer/ Reference | Pore size (m) | SIP  Y/N | Integrity Test | | 12k Mass- Stable Menge Grösse |
| Before use | After use |
| First Stage | Depth Filter B | 10099801 | Millipore L50DET6S1 | 0.7 | J | N/A | N/A | 2x  ( 4 x 16”) |
| Second Stage | Depth Filter B | 10076748 | Pall Supradisc 300PEKSC421SP | 0.2 | J | N/A | N/A | 2 x  ( 4 x 16”) |
| Third Stage | Vorfilter (Membrane-filter)b | 10077303 | Pall Fluorodyne EX AB3UEDF7PH4 | 0.2 | J | N | N | 7 x 30" |
| HCCF  Pool tank | Membrane-filterb | 10076165 | Millipore Express SHC CHGE73TS3 | 0.5/0.2 | J | Or | B | 3 x 30” |
| 10077303 | Pall Fluorodyne EX AB3UEDF7PH4 | 0.2 | J | Or | B | 3 x 30” |
| Separator Belüftungs- filter | Gasfilter | 10038581 | Pall Emflon® PFR AB05PFR2PVH4 | 0.2 | J | Or | Or | 5" |
| Filterstation Belüftungs- filter | Gasfilter | 10038581 | Pall Emflon PFR AB05PFR2PVH4 | 0.2 | J | Or | Or | 5" |
| Harvest tank aeration filter | Gasfilter | 10038589 | Pall Emflon® PFR, PTFE, AB2PFR7PVH4 | 0.2 | J | Or | Or | 20" |
| a J = Yes, N = Nein, NA = keine Angabe, O = Optional, B = Benötigt.  b The maximum permissible differential pressure per filter stage is 2,068 bar (30 psi) | | | | | | | | |

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##### Table 10: Centrate Filter Area and Flush Quantities (Pre-Harvest)

|  |  |  |  |
| --- | --- | --- | --- |
| **Purged filter** | **Rinsing path** | **Final filter Total filter area (m2)** | **Min. rinsing vol. / Area of the Final Filter**  **(L/m2 )** |
| First Stage | 16 inch depth filter | 28 | 54 |
| Second Stage | 16 inch depth filter | 40 | 54 |
| Third Stage | 16 inch depth and membrane filter | 23 | 11 |

# Appendixes

[SPT017981](https://condorportal.roche.com/condorwp/showContent.action?path=/spt017981&rendition=true): Appendix to sop022666, sop022701, sop023038 and sop023041: Product\_Name v.1.2: Media and fermentation sampling plan

# Training

Assignment of the set of rules in the training system (incl. training type) was discussed with the Learning Team, no adjustments to the assignment necessary.

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