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## 1. Product description

### Components and specifications

TCX7D Feed  
(for preparation of liquid feed from powder see Solubilization Protocol)

with 60 g/L D-glucose  
without L-glutamine  
with hypoxanthine/thymidine/nucleosides

Chemically defined  
Free of animal-derived components  
Free of proteins  
Free of growth factors

### Storage

Store protected from light at 2–8 °C. Do not freeze.

### Intended use

Intended for *in vitro* research and manufacturing processes **only**. Do not use for injection or infusion!

## 2. Background information and applications

The TCX7D Feed is a chemically defined, animal component-free feed supplement. It is developed for the use as feeding solution in large scale recombinant biopharmaceutical protein production. The feed supplement contains highly concentrated nutrients to increase the productivity of CHO cells but no lipids, hydrolysates, or growth factors. TCX7D Feed supports superior cell growth and production of recombinant proteins and antibodies in suspension culture by maintaining and extending the production capability of CHO cultures compared to batch process. Consumed substances like vitamins and amino acids are replenished to increase the protein yield by process extension. TCX7D Feed is especially well suited for CHO GS cell lines but can be used for other CHO cell lines as well when L-glutamine is supplemented.

## 3. Protocol

### 3.1 Preparations

All procedures should be carried out using sterile techniques in a biosafety cabinet.

The TCX7D Feed contains 60 g/L D-glucose, one additional sugar source and is formulated without L-glutamine. For applications requiring this amino acid, we recommend supplementation of L-glutamine prior to use. L-glutamine can be added during feed preparation or from stock solution directly into the fed-batch cultivation. For higher D-glucose concentrations, D-glucose can be added as well, either during feed preparation or from stock solutions directly into the fed-batch cultivation.

### 3.2 Culture conditions

Cultures should be maintained at 37 °C. For cultivation in an incubator, a 5% CO<sub>2</sub> atmosphere is necessary.

Parameter	Value[-]
Shaker diameter	5 cm
Shaker speed	110-185 rpm
Temperature	37°C
CO <sub>2</sub>	5%

**Table 1:** Recommended culture conditions for use of Xcell media and feed products.

Using the set-up listed in table 1, the working volume of different shake flask sizes was determined (table 2). For cell lines with a strong aggregation, baffled shakers may be used. For this setup, a reduction of the shaking speed might be necessary.

Size of shaker [mL]	Shape [-]	Working volume [mL]
125	plain, vent cap	20 - 50
250	plain, vent cap	80 - 150
500	plain, vent cap	200 - 300
1000	plain, vent cap	400 - 600

**Table 2:** Recommended culture working volumes for use of Xcell media and feed products in various shake flask sizes.

### 3.3 Instructions for use in fed-batch

- 1) Start the cultivation in batch mode, use one of Xcell's media products and L-glutamine as usual.
- 2) Daily add TCX7D Feed including a sufficient amount of D-glucose and (if using glutamine-dependent cell lines) L-glutamine or apply additional D-glucose and L-glutamine supplementation to maintain D-glucose levels of 2-3 g/L and L-glutamine concentrations of 1-3 mM during fed-batch. An exemplary feeding regime for low- and high-consuming cells is shown in table 3.

Process time [days]	TCX7D Feed per 50 mL medium	
	Low-consuming cells	High-consuming cells
0	0 mL	0 mL
1	0 mL	0 mL
2	0 mL	0.5 ml
3-5	0.5 mL	1.0 ml
From 6-end	1.75 mL	2.5 mL

**Table 3:** Example of feeding regime in a fed-batch process with low- or high-consuming cells using Xell's basis medium supplemented with 8 mM L-glutamine in 50 mL working volume shaker cultivation.

- Adjust the feeding regime according to the demand of the cell line. Increase feeding with higher growth and cell density or when nutrient limitations occur. Decrease feeding if cells show poor growth, if the pH value is decreasing dramatically, or if the amount of D-glucose is increasing.

### 3.4 Bioreactor cultivation

For best performance the inoculation density in bioreactor should be in the range of  $2\text{--}6 \times 10^5$  cells/mL in Xell medium. Suggested starting parameters for bioreactor cultivations of CHO cells using Xell medium are pH 7.0-7.2, 40% DO, and a temperature of 37 °C.

The cultivation in bioreactor under controlled pH conditions might lead to differences in cellular demands. Carefully check growth and D-glucose consumption every day. Adjust feeding to higher cell densities by carefully supplementing more TCX7D Feed and/or D-glucose and/or L-glutamine in culture in exponential and stationary cultivation phase.

## 4. Ordering information

Product	Application	Order No.
TC-42	base medium and protein production for various CHO cells; esp. CHO DHFR cell lines	509 (rInsulin) 510 (IGF) 511 (w/o GF)
TCX6D	base medium and protein production for various CHO cells	1070
TCX10D	base medium and protein production for various CHO cells; esp. for CHO GS cell lines	1100
CHOlean	base medium and protein production for various CHO cells	1140
CHO TF	transfection medium, suitable for growth, transfection/infection and production for various CHO cells; esp. for transfection	886
Basic Feed	feed supplement for CHO, HEK, hybridoma, <i>et al.</i>	1092
TCX7D Feed	Feed supplement for various CHO cells	1080

**Table 4:** CHO products by Xell

Place orders: [order@xell.de](mailto:order@xell.de)

For further information or assistance contact us.

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