Customized solutions for cell cultures.

Protocol for use
TC-42 w/ growth factor
Order No. 509 and 510

Contents

1. Product description

Components and specifications

TC-42
(for preparation of liquid media from powder see Solubilization Protocol)

without L-glutamine
without hypoxanthine/thymidine
with growth hormone

Chemically defined
Free of animal-derived components
Free of proteins

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

TC-42 is a complete chemically-defined, animal-component-free, protein-free, and ready-to-use medium developed for long-term, high-performance growth and protein yield of CHO cells and other animal cell lines. The medium supports cell growth and production of e.g. recombinant proteins and antibodies in suspension culture. The medium can be used in research or in manufacturing applications. TC-42 has been developed by Xell AG for selection, adaptation, and production with the dihydrofolate reductase – gene amplification systems. For other applications, add HT supplement prior to use.

3. Protocols

3.1 Preparations

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

TC-42 is formulated without L-glutamine. Supplementation with 6-8 mM L-glutamine prior to use is recommended.

3.2 Culture conditions

Cultures should be maintained at 37 °C. For cultivation in an incubator, a 5% CO_2 atmosphere is necessary.

Parameter	Value[-]
Shaker diameter	5 cm
Shaker speed	110-185 rpm
Temperature	37°C
CO_2	5%

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

Using the set-up listed in table 1, the working volume of different polycarbonate Erlenmeyer shake flask sizes was determined (table 2). For cell lines with strong aggregation, baffled shake flasks 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 Xell media and feed products in various shake flask sizes.

3.3 Instructions for use

3.3.1 Thawing of cells

1) Quickly thaw a vial of frozen cells in a 37 °C water bath.
2) Transfer the cells aseptically to a centrifugation tube containing 10 mL of TC-42.
3) Centrifuge cell suspension at 115×g for 5 minutes.
4) Aspirate supernatant completely and discard.
5) Resuspend the cells in 10 mL TC-42 per vial.
6) Adjust viable cell density to 5-10×10^5 cells/mL by medium addition and transfer cell suspension into an agitated or stationary cultivation system (e.g. T-75 tissue culture flask, 125 mL polycarbonate Erlenmeyer flask, or 50 mL filter tube).
7) Count the cells after 24-48 hours for assessment of cell density and viability.

8) Adjust cell density to 3–6×10⁵ cells/mL. *
9) Proceed with routine cultivation.

* Depending on the cell line, the target inoculation cell density can be lower.

3.3.2 Routine cultivation and cell expansion

1) Pre-equilibrate a sufficient amount of medium in a polycarbonate Erlenmeyer shake flask (Parameters listed in tables 1 and 2) for 1 hour. **
2) Determine viable cell density in the pre-culture.
3) Depending on the inoculation volume, remove medium from the shake flask to reach the target working volume after inoculation. Final working volume of given shaker size is listed in table 2.
4) Seed cells at a target inoculation cell density of 3×10^5 cells/mL (operational range 2–5×10⁵ cells/mL).
5) Incubate the culture according to the conditions listed in table 1.
6) Routinely passage the culture when viable cell densities between 15–40×10⁵ cells/mL are reached. Typical duration time for the culture is 3-4 days.
7) If cell density is too low or cells do not grow in adaption phase, centrifuge the culture and exchange the medium without dilution after 4 days.

** Depending on cell line, the pre-equilibration of medium might be not necessary. For some cell lines the use of 2-8°C cold culture medium directly from refrigerator was found to be beneficial. This procedure eliminates handling variations of the medium in the pre-equilibration phase of the medium.

3.3.3 Stepwise adaptation from serum-containing cultures

1) Expand the culture in serum-containing standard medium.
2) Centrifuge a sufficient number of cells for inoculation of suspension culture with 4–6×10⁵ cells/mL at 115×g for 5 minutes.
3) Resuspend cells in Xell medium (if necessary, include 6-8 mM L-glutamine) and 2 % fetal bovine serum (FBS).
4) Passage cells or change medium by centrifugation every two to four days depending on cell density.
5) Reduce serum concentration to 0.5 % after at least three passages.
6) Passage cells or change media by centrifugation every two to four days depending on cell density.
7) Reduce serum concentration to 0 % after two to four passages.
8) Continue cultures until viabilities stabilize at > 90 %.
9) Adapted cells should be inoculated at 2-5×10⁵ cells/mL in Xell medium for optimal performance. Cultures should be diluted every three or four days. Due to aggregation of CHO cells, cultures should be stirred or shaken, using spinner bottles, shaker flasks or similar cultivation systems.

3.3.4 Bioreactor cultivation

For best performance the inoculation density in bioreactor should be in the range of 4–6×10⁵ cells/mL in Xell medium. Suggested starting parameters for bioreactor cultivations of CHO cells using Xell medium are pH 7.1–7.6, 40% DO, and a temperature of 37 °C. The medium already contains Pluronic® F68, further supplementation is not necessary.

3.3.5 Freezing of cells

Cells can be frozen in TC-42 without the use of serum.

1) Choose a well-growing culture with viabilities above 90 %.
2) Prepare a freezing medium consisting of 90 % TC-42 and 10 % dimethyl sulfoxide (DMSO; cell culture grade).
3) Cool down the freezing medium to 2–8 °C.
4) Centrifuge the cells at 115×g for 5 minutes.
5) Aspirate supernatant completely.
6) Resuspend the cells in freezing medium at 1×10^7 cells/mL.
7) Rapidly transfer 1.5 mL of this suspension to sterile cryovials.
8) Place the vials in a pre-cooled (2–8 °C) freezing module and store the modules including the vials for 24 hours at -80 °C.
9) Transfer the cryovials to a –140 °C to –196 °C system for long time storage.

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