

## cQrex<sup>®</sup> Application Guidelines

Boosting your cell culture performance:  
Recommendations for evaluating cQrex<sup>®</sup> peptides and antioxidants



## Introduction

Sufficient supply of amino acids is critical for bioprocess performance. The cQrex® ingredients help to improve media formulations and bioprocesses in biopharma mammalian cell culture. They ensure a high productivity and facilitate processing by enabling the formulation of more concentrated and stable single feed media systems at neutral pH.

Some amino acids are essential for cell culture but have disadvantages such as instability or low solubility. These limitations are overcome by combining the target amino acid with another one that compensates the drawback, forming a peptide. The best-known example is L-alanyl-L-glutamine which replaces free glutamine: coupling the N-terminus of L-glutamine with L-alanine chemically stabilizes the molecule. In the case of cQrex® GY and cQrex® KC, the solubility of L-tyrosine and L-cystine is significantly increased by combining them with a highly soluble amino acid, such as glycine or L-lysine.

As functionally superior forms of amino acids, cQrex® peptides can be used to fully or partially substitute them in media. The full substitution of L-glutamine with a suitable peptide is an easy task because the amino acid is usually added to the medium separately. In the case of L-tyrosine and L-cystine peptides, we recommend an initial evaluation by adding them to the current feed or basal medium in a range of concentrations. As they are highly soluble, the concentrations in the bioreactor can be increased without risking precipitation.

Once the ideal concentrations are confirmed, media reformulation can be considered as a next step to further improve outcomes. For example, it may be considered to partially or fully reduce the concentrations of the respective amino acids and replace them with suitable peptide concentrations.

Depending on the product and concentrations required, it may be preferred to either use the cQrex® peptides in stock solutions (water or medium) to evaluate the effect of different concentrations, or to add them directly to a medium. When working with dry powder media or liquid media, they can be directly added and rehydration protocols do not need to be adapted. Subsequent sterile filtration is necessary in all cases. Suggestions for stock solutions as well as other product information can be found in the following product sections.

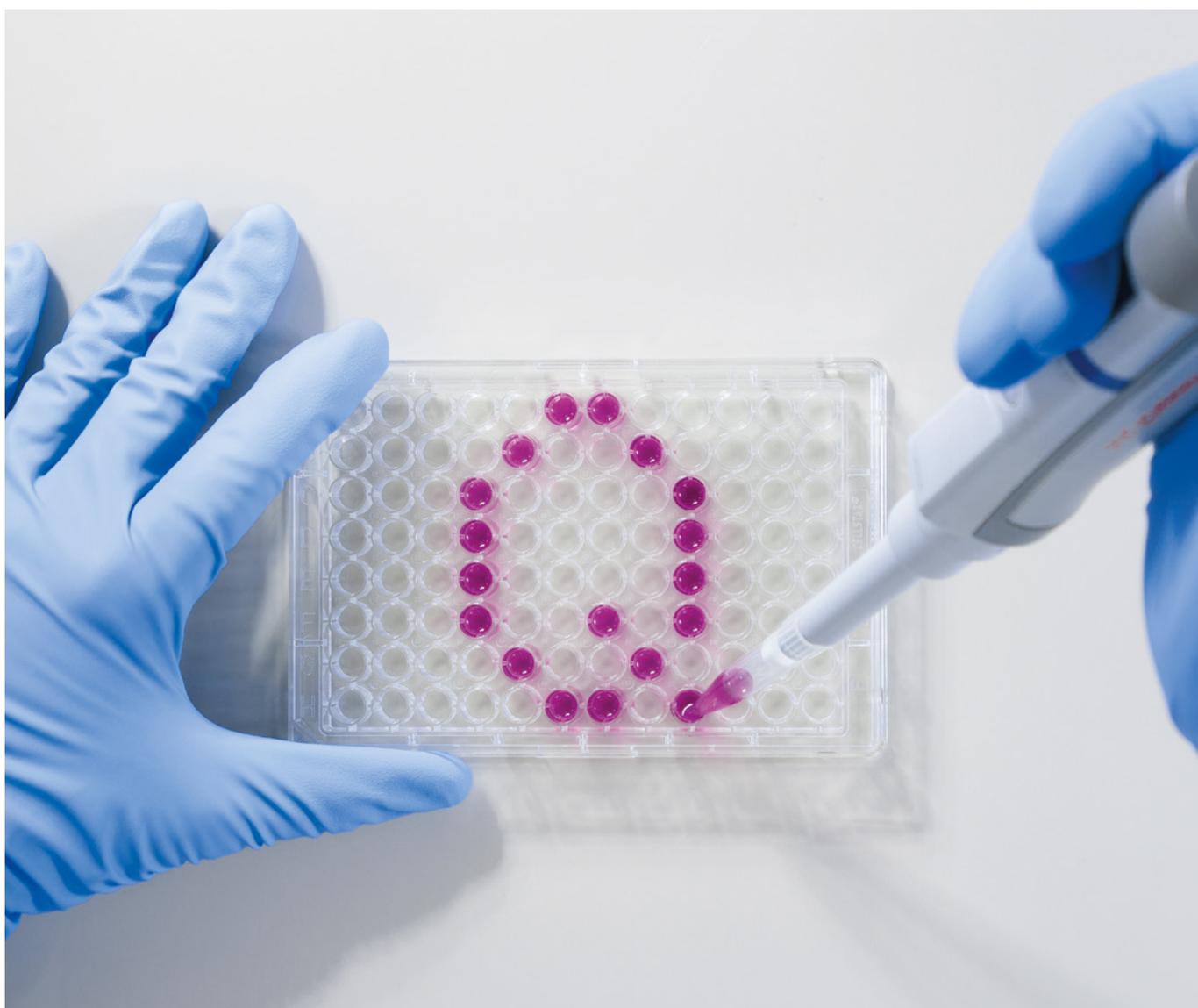
The recommendations given in the following pages are general guidelines. We suggest testing several concentrations before selecting the one which provides you with the best results.

All products in the cQrex® portfolio are non-animal derived, chemically defined single components supplied as pure substances in crystalline form.

Please contact us if you have questions and we will be glad to provide you with further guidance.

## Overview of cQrex® ingredients

PRODUCT NAME	MOLECULAR WEIGHT	SOLUBILITY IN WATER (20 °C, PH 7)		SAMPLE QUANTITY
<b>cQrex® AQ</b> L-Alanyl-L-glutamine	217.23 g/mol	720 g/L	3314 mM	10 g
<b>cQrex® GQ</b> Glycyl-L-glutamine hydrate	221.23 g/mol	154 g/L	696 mM	10 g
<b>cQrex® GY</b> Glycyl-L-tyrosine dihydrate	274.27 g/mol	34 g/L	124 mM	10 g
<b>cQrex® AY</b> L-Alanyl-L-tyrosine trihydrate	306.31 g/mol	21 g/L	69 mM	10 g
<b>cQrex® AC</b> N,N'-di-L-Alanyl-L-cystine H <sub>2</sub> O	400.48 g/mol	14 g/L	34 mM	6 g
<b>cQrex® KC</b> N,N'-di-L-Lys-L-CySS 2HCl H <sub>2</sub> O	587.57 g/mol	>588 g/L	>1000 mM	10 g
<b>cQrex® AKG</b> Alpha-Ketoglutaric acid	146.07 g/mol	>1000 g/L	>6846 mM	5 g



## cQrex® AQ L-ALANYL-L-GLUTAMINE

**Molar mass:** 217.23 g/mol

**Effect:** Stabilized form of L-glutamine, prevents glutamine decomposition that otherwise releases free ammonia in media or feeds. Ammonia can be detrimental to cell viability and negatively influence protein quality. cQrex® AQ supplemented cultures trend towards higher cell densities compared to cultures using free glutamine or other glutamine peptides.

**Solubility:** 720 g/L (3314 mM) in water, media or buffer at neutral pH.

**Mode of action:** Formation of a peptide bond between L-alanine and the N-terminus of L-glutamine prevents chemical degradation. Biologically, a fast uptake rate of cQrex® AQ compared to GQ was observed<sup>1</sup>. This can be beneficial for cells with a high glutamine demand. After import, the peptide is enzymatically hydrolyzed into L-alanine and L-glutamine. cQrex® AQ was found to be useful for a wide variety of mammalian cells. If the cell line is sensitive to L-alanine, testing cQrex® GQ is recommended.

**Use:** cQrex® AQ can be used in combination with every glutamine-free medium. It can be dissolved directly in the liquid medium or added to the dry powder media before rehydration. Rehydration protocols do not need to be adapted. To initially determine the suitable concentration, highly concentrated stock solutions can otherwise be prepared as described below.

**Cell line:** Recommended for mammalian cell lines or primary cells. Not recommended for cell lines using a glutamine-dependent selection system.

**Recommended concentration:** Equimolar compared to the recommended molar concentration of free glutamine, typically between 4 mM and 8 mM (e.g. substitute 4 mM free glutamine with 4 mM of cQrex® AQ). If needed, reduce the concentration of free L-alanine by the molar concentration added via the peptide (e.g. 4 mM cQrex® AQ will become 4 mM L-alanine and 4 mM L-glutamine). If slow growth is observed after switching to the peptide, perform two passages with partial substitution (e.g. 3 mM cQrex® AQ and 1 mM of free glutamine).

**Stock solutions:** The recommended concentration is 200 mM, which is equivalent to many commercial formulations of stabilized glutamine.

### Stock solution (200 mM) 100 mL in medium or water

INGREDIENT	AMOUNT
cQrex® AQ L-Alanyl-L-glutamine	4.345 g
Water	Fill up to 100 mL
pH after dissolution	~5.8
Dissolution time	~5 min

A magnetic stirrer should be used for small-scale reconstitution up to 5 L. Adjust pH with 1 M NaOH if needed. Sterile filter after preparation, use a sterile bottle. The dissolution time depends on the concentration of the solution: Lower concentrations result in faster dissolution.

**Storage stability:** Storage at 2–8 °C or at ambient temperature, avoid direct sunlight. cQrex® AQ is stable in stock solution and media for at least two months at ambient temperature.

**Note:** Solubility is temperature dependent. Precipitation can occur during cold storage. Please allow sufficient time at room temperature for re-equilibration prior to use. 30 minutes are typically sufficient at small scale. Use of temperature bath at 37 °C and agitation reduce the time needed for re-equilibration.

## cQrex® GQ GLYCYL-L-GLUTAMINE HYDRATE

**Molar mass:** 221.23 g/mol

**Effect:** Stabilized form of L-glutamine, prevents glutamine decomposition that otherwise releases free ammonia to media or feeds. Ammonia can be detrimental to cell viability and negatively influence protein quality. Compared to cQrex® AQ or other alanine-based forms of stabilized glutamine, cQrex® GQ supplemented cultures tend towards lower cell densities at identical or elevated titer levels. Peptide-supplemented cultures often show lower levels of lactate and ammonia compared to cultures using free glutamine.

**Solubility:** 154 g/L (696 mM) in water, media or buffer at neutral pH.

**Mode of action:** Formation of a peptide bond between glycine and the N-terminus of L-glutamine prevents chemical degradation. Biologically, a slower but more consistent uptake rate of cQrex® GQ compared to AQ was observed<sup>1</sup>. This can help to reduce waste metabolites. After import, the peptide is enzymatically hydrolyzed into glycine and L-glutamine. cQrex® GQ is preferable to cQrex® AQ or other alanine-based forms of stabilized glutamine if an accumulation of alanine is observed.

**Use:** cQrex® GQ can be used in combination with every glutamine-free medium. It can be dissolved directly in the liquid media or added to the dry powder media before rehydration. Rehydration protocols do not need to be adapted. To initially determine the suitable concentration, highly concentrated stock solutions can otherwise be prepared as described below.

**Cell line:** Recommended for mammalian cell lines or primary cells. Not recommended for cell lines using a glutamine-dependent selection system.

**Recommended concentration:** Equimolar compared to the recommended molar concentration of free glutamine, typically between 4 mM and 8 mM (e.g., substitute 4 mM free glutamine 4 mM of cQrex® GQ). If needed, reduce the concentration of free glycine by the molar concentration added via the peptide (e.g., 4 mM cQrex® GQ will become 4 mM glycine and 4 mM L-glutamine). If slow growth is observed after switching to the peptide, perform two passages with partial substitution (e.g., 3 mM cQrex® GQ and 1 mM of free glutamine).

**Stock solutions:** The recommended concentration is 200 mM, which is equivalent to many commercial formulations of stabilized glutamine.

**Stock solution (200 mM) 100 mL in medium or water**

INGREDIENT	AMOUNT
cQrex® GQ Glycyl-L-glutamine hydrate	4.425 g
Water	Fill up to 100 mL
pH after dissolution in water	~5.8
Dissolution time	~10 min

A magnetic stirrer should be used for small-scale reconstitution up to 5 L. Adjust pH with 1 M NaOH if needed. Sterile filter after preparation, use a sterile bottle. For minimal dilution, stock solutions up to 600 mM are possible. The dissolution time depends on the concentration of the solution: Lower concentrations result in faster dissolution.

**Storage stability:** Storage at 2–8 °C or at ambient temperature, avoid direct sunlight. cQrex® GQ is stable in stock solutions and media for at least two months at ambient temperature.

**Note:** Solubility is temperature dependent. Precipitation can occur during cold storage. Please allow sufficient time at room temperature for re-equilibration prior to use. 30 minutes are typically sufficient at small scale. Use of temperature bath at 37 °C and agitation reduce the time needed for re-equilibration.

## cQrex® GY GLYCYL-L-TYROSINE DIHYDRATE

**Molar mass:** 274.27 g/mol

**Effect:** Highly soluble and bioavailable form of L-tyrosine, ideally used as part of a pH neutral feed medium. Eliminates the risks associated with using a separate high-pH feed medium for tyrosine dissolution. For best performance, it can be considered to reduce the concentration of free glycine in the feed medium to account for the glycine added via the peptide.

**Solubility:** 34 g/L (124 mM) in water, media or buffer at neutral pH.

**Mode of action:** Coupling glycine to L-tyrosine via a peptide bond results in a chemically stable peptide with a higher solubility than free tyrosine. The peptide is imported by the cell, then enzymatically hydrolyzed to glycine and L-tyrosine. Uptake was found to be very efficient<sup>1</sup>.

**Use:** cQrex® GY can be dissolved directly in the liquid media or added to the dry powder media before rehydration. Rehydration protocols do not need to be adapted. To initially determine the suitable concentration, highly concentrated stock solutions can otherwise be prepared as described below. cQrex® GY can be combined with other cQrex® products. Typically, no adaption is needed.

**Cell line:** Useful for all cell lines, including those with a glutamine-dependent selection system.

**Recommended concentration:** Basal media typically contain between 0.2 and 0.6 mM tyrosine as disodium salt. Feed media concentrations typically range from 5 to 50 mM. Due to the high solubility, cQrex® GY can support neutral pH feeds up to 124 mM and replace all other tyrosine sources.

**Stock solutions:** the recommended concentration is 110 mM.

### Stock solution (110 mM) 100 mL in medium or water

INGREDIENT	AMOUNT
cQrex® GY Glycyl-L-tyrosine dihydrate	3.017 g
Water	Fill up to 100 mL
pH after dissolution in water	~5.5
Dissolution time	~60 min

A magnetic stirrer should be used for small-scale reconstitution up to 5 L. Adjust pH with 1 M NaOH if needed. Sterile filter after preparation, use a sterile bottle. The dissolution time depends on the concentration of the solution: Lower concentrations result in faster dissolution.

**Storage stability:** Storage at 2–8 °C or at ambient temperature, avoid direct sunlight. cQrex® GY is stable in stock solutions and media for at least two months at ambient temperature.

**Note:** Solubility is temperature dependent. Precipitation can occur during cold storage. Please allow sufficient time at room temperature for re-equilibration prior to use. 30 minutes are typically sufficient at small scale. Use of temperature bath at 37 °C and agitation reduce the time needed for re-equilibration.

## cQrex® AY L-ALANYL-L-TYROSINE TRIHYDRATE

**Molar mass:** 306.31 g/mol

**Effect:** Highly soluble and bioavailable form of L-tyrosine, ideally used as part of a pH-neutral feed medium. Eliminates the risks associated with using a separate high-pH feed medium for tyrosine dissolution. For best performance, it may be considered to reduce the concentration of free L-alanine in the medium to account for the L-alanine added via the peptide.

**Solubility:** 21 g/L (69 mM) in water, media or buffer at neutral pH.

**Mode of action:** Coupling L-alanine to L-tyrosine via a peptide bond results in a chemically stable peptide with a higher solubility than free tyrosine. The peptide is imported by the cell, then enzymatically hydrolyzed to L-alanine and L-tyrosine.

**Use:** cQrex® AY can be dissolved directly in the liquid media or added to the dry powder media before rehydration. Rehydration protocols do not need to be adapted. To initially determine the suitable concentration, highly concentrated stock solutions can otherwise be prepared as described below. cQrex® AY can be combined with other cQrex® products. Typically, no adaption is needed.

**Cell line:** Useful for all cell lines, including those with a glutamine-dependent selection system.

**Recommended concentration:** Basal media typically contain between 0.2 and 0.6 mM tyrosine as disodium salt. Feed media concentrations typically range from 5 to 50 mM. Due to the high solubility, cQrex® AY can support neutral pH feeds up to 69 mM and replace all other tyrosine sources.

**Stock solutions:** The recommended concentration is 50 mM.

**Stock solution (50 mM) 100 mL in medium or water**

INGREDIENT	AMOUNT
cQrex® AY L-Alanyl-L-tyrosine trihydrate	1.532 g
Water	Fill up to 100 mL
pH after dissolution in water	~5.5
Dissolution time	~240 min

A magnetic stirrer should be used for small-scale reconstitution up to 5 L. Adjust pH with 1 M NaOH if needed. Sterile filter after preparation, use a sterile bottle. The dissolution time depends on the concentration of the solution: Lower concentrations result in faster dissolution.

**Storage stability:** Storage at 2–8 °C or at ambient temperature, avoid direct sunlight. cQrex® AY is stable in stock solutions and media for at least two months at ambient temperature.

**Note:** Solubility is temperature dependent. Precipitation can occur during cold storage. Please allow sufficient time at room temperature for re-equilibration prior to use. 30 minutes are typically sufficient at small scale. Use of temperature bath at 37 °C and agitation reduce the time needed for re-equilibration.

## cQrex® AC N,N'-DI-L-ALANYL-L-CYSTINE H<sub>2</sub>O

**Molar mass:** 400.48 g/mol

**Effect:** Highly soluble and bioavailable form of L-cystine which avoids the risk of free L-cysteine oxidation to L-cystine. It can be used to increase the concentration of L-cysteine/L-cystine in basal media or prepare highly concentrated, pH- neutral feeds. When added to the basal media, it can prevent precipitation caused by feeding excess L-cysteine and/or L-cystine.

**Solubility:** 14 g/L (34 mM) in water, media or buffer at neutral pH.

**Mode of action:** Coupling two moles of L-alanine to L-cystine via a peptide bond results in a disulfide coupled dipeptide dimer with a higher solubility than free L-cystine. The peptide is eventually reduced and hydrolyzed to free L-alanine and L-cysteine in the cell. In the cell culture medium, cQrex® AC acts as a stabilizer to prevent L-cystine precipitation caused by L-cysteine oxidation in the medium.

**Use:** cQrex® AC can be dissolved directly in the liquid media or added to the dry powder media before rehydration. Rehydration protocols do not need to be adapted. To initially determine the suitable concentration, highly concentrated stock solutions can otherwise be prepared as described below.

**Cell line:** Useful for all cell lines, including those with a glutamine-dependent selection system.

**Recommended concentration:** Basal media typically contain between 0.2 and 0.8 mM L-cysteine/L-cystine species. Feed media concentrations typically range from 5 to 30 mM. Due to the high solubility, cQrex® AC can support neutral pH feeds up to 34 mM. Full replacement of L-cysteine/L-cystine in the feed medium is normally possible. However, L-cysteine in the basal medium should not be replaced. Instead, cQrex® AC can be added to existing basal media formulation in a range of concentrations to screen for beneficial effects in various media backgrounds and cell lines. In some cases, it can be beneficial to use a molar ratio of cQrex® AC to free L-cysteine between 1:1 and 5:1 in the medium.

**Stock solutions:** Stock solutions of up to 34 mM can be prepared at neutral pH. However, in most cases, it is preferable to directly add the product to the basal and/or feed medium. The following example describes the preparation of a 20 mM stock solution.

### Stock solution (20 mM) 100 mL in medium or water

INGREDIENT	AMOUNT
cQrex® AC N,N'-di-L-Alanyl-L-cystine H <sub>2</sub> O	0.801 g
Water	Fill up to 100 mL
pH after dissolution in water	~5.5
Dissolution time	~120 min

A magnetic stirrer should be used for small-scale reconstitution up to 5 L. Adjust pH with 1 M NaOH if needed. Sterile filter after preparation, use a sterile bottle. The dissolution time depends on the concentration of the solution: Lower concentrations result in faster dissolution.

**Storage stability:** Storage at 2–8 °C or at ambient temperature, avoid direct sunlight. cQrex® AC is stable in stock solutions and media for at least two months at ambient temperature.

**Note:** Solubility is temperature dependent. Precipitation can occur during cold storage. Please allow sufficient time at room temperature for re-equilibration prior to use. 30 minutes are typically sufficient at small scale. Use of temperature bath at 37 °C and agitation reduce the time needed for re-equilibration.

## cQrex® KC N,N'-DI-L-LYS-L-CYSS 2HCL H<sub>2</sub>O

**Molar mass:** 587.57 g/mol

**Effect:** Highly soluble and bioavailable form of L-cystine which avoids the risk of free L-cysteine oxidation to L-cystine. It can be used to increase the concentration of L-cysteine/L-cystine in basal media or prepare highly concentrated, pH-neutral feeds. When added to the basal media, it can prevent precipitation caused by feeding excess L-cysteine and/or L-cystine.

**Solubility:** >588 g/L (>1000 mM) in water, media or buffer at neutral pH.

**Mode of action:** Coupling two moles of L-lysine to L-cystine via a peptide bond results in a disulfide coupled dipeptide dimer with a higher solubility than free L-cystine. The peptide is eventually reduced and hydrolyzed to free L-lysine and L-cysteine in the cell. In the cell culture medium, cQrex® KC acts as a stabilizer to prevent L-cystine precipitation caused by L-cysteine oxidation in the medium.

**Use:** cQrex® KC can be dissolved directly in the liquid media or added to the dry powder media before rehydration. Rehydration protocols do not need to be adapted. To initially determine the suitable concentration, highly concentrated stock solutions can otherwise be prepared as described below.

**Cell line:** Useful for all cell lines, including those with a glutamine-dependent selection system.

**Application guideline:** In most cases, it is preferable and more user-friendly to directly add the product to the powder basal and/or feed medium, for example to reach high concentrations. Otherwise, cQrex® KC can be added as powder (media needs to be sterile filtered afterwards) or as a highly concentrated (>1000 mM) and sterile stock solution to liquid basal or feed media. Any addition of cQrex® KC has no large influence on media pH and osmolarity thereof. Additionally, existing rehydration protocols and pH adjustment steps do not need to be changed.

Recommendations differ according to the use case.

### 1. Use cQrex® KC as a booster in basal or feed media:

cQrex® KC can be added to existing basal and feed media formulation in a range of concentrations to screen for beneficial effects in various media backgrounds and cell lines. L-cysteine/L-cystine in the basal medium should not be fully replaced.

Basal media typically contain between 0.2 and 0.8 mM and feed media between 4 mM and 20 mM L-cysteine/L-cystine species. In some cases, it can be beneficial to use a molar ratio of cQrex® KC to free L-cysteine between 1:1 and 5:1 in the basal and/or feed medium.

### 2. Full replacement of L-cysteine/L-cystine by cQrex® KC in a feed medium:

- Single-feed systems can be reformulated by replacing the original L-cysteine/L-cystine with an equimolar amount of cQrex® KC (e.g., 2 mM L-cysteine are equivalent to 1 mM cQrex® KC). In some cases, it can be more beneficial to use a molar ratio of cQrex® KC to free L-cysteine between 1:1 and 5:1 in the feed medium.
- cQrex® KC can be used to make a single-feed system out of a (commercial) two-feed system. The desired concentration depends on the required ratio of other amino acids (feed A) to L-cysteine/L-cystine and L-tyrosine, originally delivered via the second feed (feed B). With known amino acid concentrations of feed B, the desired concentration of cQrex® KC to be added to feed A can be calculated based on the original feed regime of the two-feed system. Usually, the supplied volume of feed B is 10% (v/v) of the supplied volume of feed A (e.g., 0.3% (v/v) feed B and 3% (v/v) feed A are fed daily). Otherwise, add cQrex® KC at concentrations of 10–15 mM to the single-feed media. Using a pH-neutral single-feed system, the supply of the desired L-tyrosine amount, usually 15–20 mM, can be achieved by working with highly soluble L-tyrosine peptides\* such as cQrex® GY or cQrex® AY or alternative soluble forms of L-tyrosine.

When using L-cystine peptides such as cQrex® KC or cQrex® AC, cell growth can be slower compared to using free amino acids supplied by the original second feed B. The hydrolysis and/or cellular metabolism of cystine peptides is slower compared to L-tyrosine peptides such as cQrex® GY and cQrex® AY.<sup>1</sup> Therefore, consider adding 1–2 mM cQrex® KC to the basal medium and/or creating a molar ratio of cQrex® KC to free L-cysteine between 1:1 and 5:1 with the addition of free L-cysteine to the feed medium.

\*FTO limitations for specific formulation options with peptides may apply in some countries – contact us for more information.

## cQrex® KC N,N'-DI-L-LYS-L-CYSS 2HCL H<sub>2</sub>O

**Stock solutions:** stock solutions of 500 mM can be prepared at neutral pH.

### Stock solution (500 mM) 100 mL in medium or water

INGREDIENT	AMOUNT
cQrex® KC N,N'-di-L-Lys-L-CySS 2HCl H <sub>2</sub> O	29.379 g
Water	Fill up to 100 mL
pH after dissolution in water	~5.2
Dissolution time	~5 min

A magnetic stirrer should be used for small-scale reconstitution up to 5 L. Adjust pH with 1 M NaOH if needed. Usually, the addition of the acidic cQrex® KC stock solution will be buffered by the media buffer system. Sterile filter after preparation, use a sterile bottle. The dissolution time depends on the concentration of the solution: Lower concentrations result in faster dissolution.

**Storage stability:** Storage at 2–8 °C or at ambient temperature, avoid direct sunlight. cQrex® KC is stable in stock solutions and media for at least two months at ambient temperature.

**Note:** solubility is temperature dependent. Precipitation can occur during cold storage. Please allow sufficient time at room temperature for re-equilibration prior to use. 30 minutes are typically sufficient at small scale. Use of temperature bath at 37 °C and agitation reduce the time needed for re-equilibration.

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**General note:** When using highly soluble peptides as alternatives for amino acids like L-cysteine/L-cysteine and L-tyrosine, carrier amino acids such as L-lysine, glycine or L-alanine are also added to the basal and/or feed medium and are available intracellularly once the peptides are metabolized. Therefore, if needed, consider adjusting L-lysine, glycine or L-alanine concentrations in the basal or feed medium depending on the peptides that are chosen to work with.

## cQrex® AKG ALPHA-KETOGLUTARIC ACID

**Molar mass:** 146.07 g/mol

**Effect:** cQrex® AKG is a metabolite derived from L-glutamine which is part of the TCA cycle. It also acts as a scavenger against reactive oxidative species<sup>2</sup>. By protecting the cell and the product from oxidative stress, higher titer and promoted cell growth can be achieved. For insect cells, Carinhas *et al.* reported a 6 to 7 fold increase in baculovirus yield<sup>3</sup>.

**Solubility:** >1000 g/L (>6846 mM) in water, media or buffer at neutral pH.

**Mode of action:** Components present in DMEM and other media can undergo oxidation and form H<sub>2</sub>O<sub>2</sub> or other oxidation products. cQrex® AKG can react with these components, decreasing levels of H<sub>2</sub>O<sub>2</sub>. AKG is converted to succinate in the process<sup>2</sup>.

**Use:** cQrex® AKG can be dissolved directly in the liquid media or added to the dry powder media before rehydration. Rehydration protocols do not need to be adapted. To initially determine the suitable concentration, highly concentrated stock solutions can otherwise be prepared as described below. In order to maximize the protective effect, an addition to the basal medium is highly recommended. This can be a new formulation or a commercial off-the-shelf medium.

**Cell line:** Useful for all cell lines, including insect cells.

**Recommended concentration:** 2–4 mM in the basal medium is recommended.

**Preparation of stock solutions:** The recommended concentration is 200 mM.

### Stock solution (200 mM) 100 mL in water

INGREDIENT	AMOUNT
cQrex® AKG Alpha-Ketoglutaric acid	2.921 g
Water	dissolve in 50mL, adjust pH with 1M NaOH, top up with water
1 M NaOH	Add approx. 40mL for pH 7.2
pH before/after dissolution/after pH adjustment	5.2/1.7/7.2
Dissolution time	~1 min

A magnetic stirrer should be used for small-scale reconstitution up to 5 L. Adjust pH with 1 M NaOH if needed. Sterile filter after preparation, use a sterile bottle.

**Storage stability:** Storage at 2–8 °C or at ambient temperature, avoid direct sunlight. cQrex® AKG is stable in stock solutions and media for at least two months at ambient temperature.

#### References:

- 1: Sanchez-Kopper *et al.* *AMB Expr* (2016) 6:48
- 2: L.H. Long, B. Halliwell/*Biochemical and Biophysical Research Communications* 406 (2011) 20–24
- 3: N. Carinhas *et al.*/*Metabolic Engineering* 12 (2010) 39–52

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