



Xuri IL-2

Product Support Documentation

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1 Introduction

Product code

29062789 Xuri™ IL-2 Xuri IL-2 10 µg

29062790 Xuri IL-2 1 mg

Important

Read these instructions carefully before using the products.

Intended use

Xuri IL-2 (Product codes 29062789, 29062790) is intended for the *ex vivo* expansion of human T lymphocytes derived from donor blood or bone marrow. Any claims within this document are strictly linked to the intended use and are not valid beyond.

The products are not intended for any therapeutic or diagnostic use in humans or animals. Do not use internally or externally in humans or animals.

Safety

For use and handling of the products in a safe way, refer to the Safety Data Sheets.



WARNING

Handling blood: Take necessary precautions when handling blood and follow local risk assessment guidelines.

User responsibility

All data and performance claims in this document are subject to the specific set up of the experiments by which they have been concluded. Each user must make an independent judgment on the validity of the data as well as perform testing procedures for the validation of their own application. Cytiva cannot guarantee the successful outcome of any experiments conducted with Cytiva products in the user's hands.

Product description

Xuri IL-2 is a human recombinant interleukin-2 for the promotion of activated human T lymphocyte expansion. Xuri IL-2 is provided as lyophilized powder to be reconstituted prior to use. The product is for single use only.

Composition

Xuri IL-2 is produced by recombinant expression of the human sequence for interleukin-2 in *Escherichia coli*. The general sequence for IL-2 is publicly available (<http://www.uniprot.org/uniprot/P60568>). Minor modifications to the sequence for improved expression or activity might apply but are proprietary.

The following excipients were used in the manufacturing process:

Chemical	CAS-NO
Sodium dodecyl sulfate (SDS) solution	15-121-3
Mannitol	69-65-8
Monosodium phosphate	7558-80-7
Disodium phosphate	7558-79-4

Animal component free

None of the components of Xuri IL-2 or those used in the manufacturing process are derived or extracted from animal or human origin.

2 Production quality

Country of origin

Xuri IL-2 is manufactured in Argentina for Cytiva.

Production standard and quality assurance

All aspects of manufacturing, testing and release of Xuri IL-2 fall under the control of a Quality Management System certified to ISO 9001:2008. Xuri IL-2 is manufactured under a GMP license certified and regularly audited by the Instituto Nacional de Medicamentos according to the regulations in force in the Argentine Republic.

Sterilization and lyophilization

Prior to lyophilization Xuri IL-2 is manufactured in liquid form containing sodium dodecyl sulphate, mannitol, anhydrous monosodium phosphate and anhydrous disodium phosphate. It is then filtered at 0.2 µm under clean room conditions according to current Good Manufacturing Practices (cGMP) following ISO 13408-2 (aseptic filtration). Lyophilization is performed under sterile conditions as per ISO 13408-3.

3 Product quality specifications

USP<1043> criteria

Xuri IL-2 meets USP<1043> 'ancillary materials for cell, gene, and tissue-engineered products', within the responsibilities applicable to a supplier. Cytiva cannot fulfil USP<1043> in regards of application and therapy specific aspects (e.g. use in finished therapeutic, assessment of removal from a finished therapeutic and possible biocompatibility, cytotoxicity or adventitious agent testing).

Product release criteria

Xuri IL-2 is tested and released under the following criteria:

- Appearance to be white powder
- Positive identification by Western Blot with an IL-2 epitope specific antibody
- Specific activity by HT-2 cell based bio assay against Proleukin to be within the range of 11.5–26.0 MIU¹/mg. This is performed prior to lyophilization
- Molecular weight by SDS PAGE followed by Coomassie™ blue detection to be within the range of 14 kDa–16 kDa
- Purity by non-reducing SDS PAGE followed by silver staining and additionally by reversed phase HPLC analysis both to be at a value of ≥95.0%
- Contamination with host cell-derived proteins (E. coli) as detected by dot plot analysis is to be ≤0.6%
- Bacterial endotoxins as measured by standard LAL test to be <25 EU/mg
- Bioburden by incubation and testing for microorganisms to be negative

Specific Activity and Final Weight

The specific activity that forms the release criteria for Xuri IL-2 is derived from a HT-2 cell based against a Proleukin standard and is performed prior to lyophilization. The final product mass is determined post lyophilization following re-suspension by ELISA. For the 1 mg unit product, the product and assay variation falls within a specification of 11.5–26.0 MIU/vial. For the 10 µg unit product, the current specification is that there shall be at least 1×10^4 IU of activity per vial. This specification will be detailed upon completion of an ongoing validation.

¹ million international units

4 Raw material quality

Supplier qualification

Raw material suppliers are qualified and monitored within the control of the quality management system (see [Production standard and quality assurance, on page 5](#)). Components used in the production of Xuri IL-2 are at least cell culture grade but aimed for USP and EP grade.

5 Validation

Validation of the product was performed in accordance with Cytiva internal QMS requirements following ISO 9001:2008. The procedure serves to demonstrate the quality and suitability of the product to the application described in the intended use.

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5.1 HT-2 Proliferation Studies

Requirement

Xuri IL-2 shall support the expansion of HT-2 cells.

Experimental set up

HT-2 cells (obtained from ATCC as HT-2 clone A5E) were thawed and maintained in medium containing a commercially available IL-2 until the viability was 85% or above to establish a base line. Subsequently the cells were passaged and maintained in medium containing 200 IU/mL Xuri IL-2 for 7 days. Fresh medium containing 200 IU/mL Xuri IL-2 was added on a daily basis to keep the cell concentration at 3×10^5 to 9×10^5 cells/mL. Samples were taken to determine cell number and viability in triplicates on a daily basis.

Data

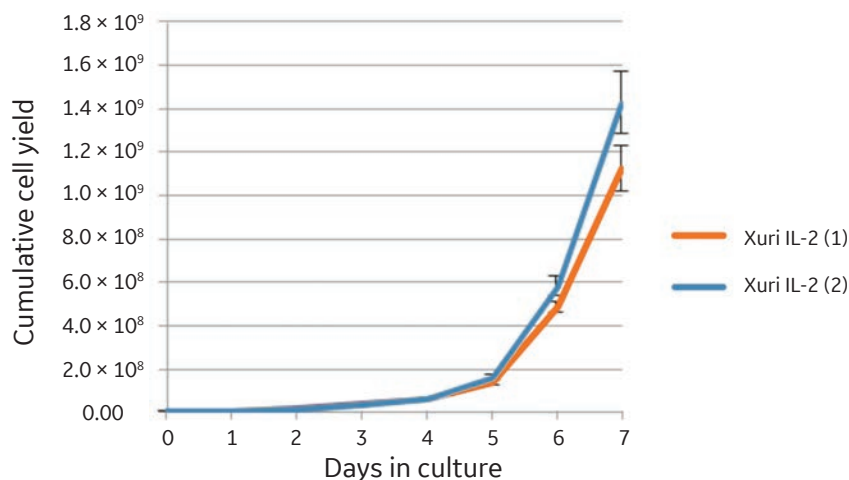


Figure 5.1: Cumulative cell yield of HT-2 cells cultivated with two batches of Xuri IL-2 (1, 2) based on triplicates for each time point.

Result

Xuri IL-2 can support the expansion of HT-2 cells under the cultivation conditions as suggested by the American Type Culture Collection Organization (www.atcc.org).

5.2 HT-2 Viability Studies

Requirement

HT-2 cells grown with Xuri IL-2 shall maintain a viability of at least 85%.

Experimental set up

HT-2 cells (obtained from ATCC as HT-2 clone A5E) were thawed and maintained in medium containing a commercially available IL-2 until the viability was 85% or above to establish a base line. Subsequently the cells were passaged and maintained in medium containing 200 IU/mL Xuri IL-2 for 7 days. Fresh medium containing 200 IU/mL Xuri IL-2 was added on a daily basis to keep the cell concentration at 3×10^5 to 9×10^5 cells/mL. Samples were taken to determine cell number and viability in triplicates on a daily basis.

Data

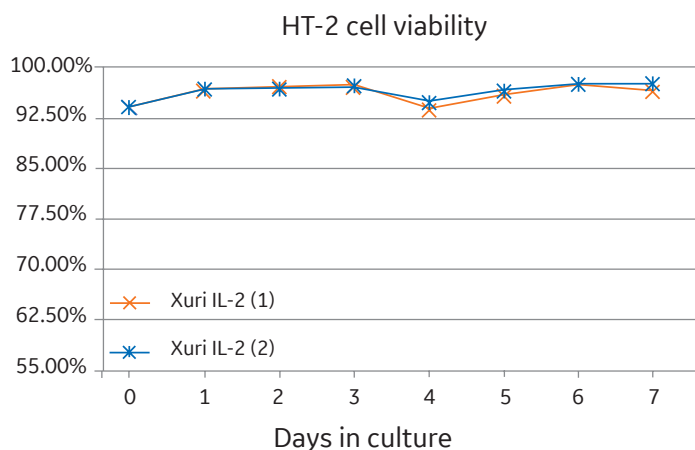


Figure 5.2: Cell viability of HT-2 cells cultivated with two batches of Xuri IL-2 (1, 2) based on triplicates for each time point.

Result

Xuri IL-2 can maintain HT-2 at high viability under the cultivation conditions as suggested by the American Type Culture Collection Organization (www.atcc.org).

5.3 T Lymphocyte Proliferation Studies

Requirement

Xuri IL-2 shall support the proliferation of human T lymphocytes from a healthy donor during a 14 day standard expansion procedure. Due to donor related variation in the starting cell population we monitored T helper cells (CD4+ T cells) and cytotoxic T cells (CD8+ T cells) for their stage of differentiation and cell senescence.

The expression patterns of the cell surface markers CD27 and CD28 give an indication of the differentiation state of T cells. Naive or early differentiated T cells are CD27+/CD28+, effector T cells are CD27+/CD28- or CD27-/CD28+ and late effectors or 'aged' T cells are CD27-/CD28- (Appay V et al, Nat. Med. 2002). Continuous activation and proliferation can drive T cells to a state of senescence, which can be detected by the expression of the cell surface marker CD57 (Brenchley JM et al, Blood 2003).

Experimental set up

Frozen human peripheral blood mononuclear cells were initially cultivated in static cell culture (T225 flasks) with X-VIVOX-VIVO™ 10 (Lonza) and 5% human serum (heat inactivated) plus 200 IU/mL Xuri IL-2. Cells were concurrently cultured with two different batches of Xuri IL-2 and activated using antibody coated beads. Cells were counted from day three on and expanded until they passed the number needed for seeding in a 2 L Cellbag (5×10^8). After 5 days in static culture, cells were transferred and cultivated on a XuriXuri W5 Cell Expansion System for an additional 9 days under perfusion. The perfusion rates were set to ensure that ammonium levels were maintained below 2.0 mmol/L, lactate levels were maintained below 2.0 g/L, and glucose levels were above 1.0 g/L. Media composition and Xuri IL-2 concentration were kept the same as in static culture. Cell count and viability analysis were performed each day. Mean of viable cells was determined on a Nucleocounter over two measurements of viable cells. Viable cell counts were calculated by subtracting non-viable cell count for each setup. T cells were phenotyped by flow cytometric analysis at day 0, 10 and 14.

Data

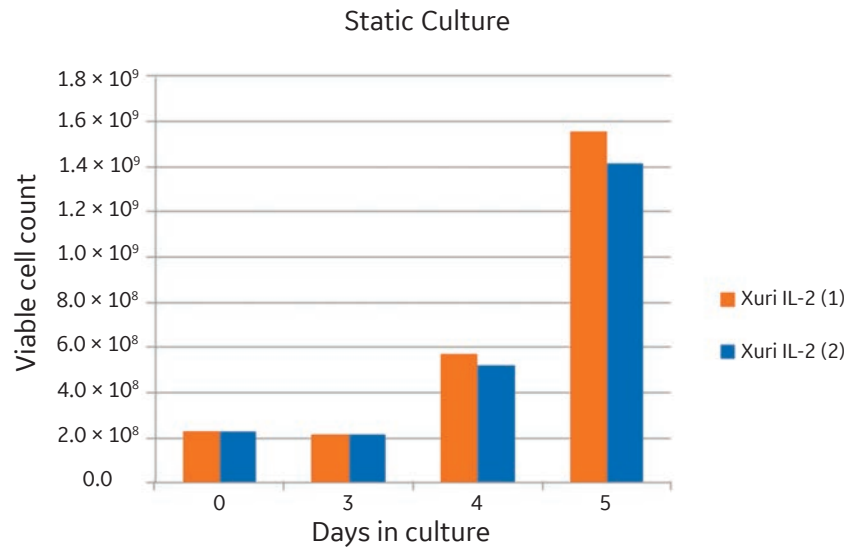


Figure 5.3: Viable cell counts of human primary T lymphocytes cultivated with two batches of Xuri IL-2 (1, 2) in static T225 flask culture over 5 days.

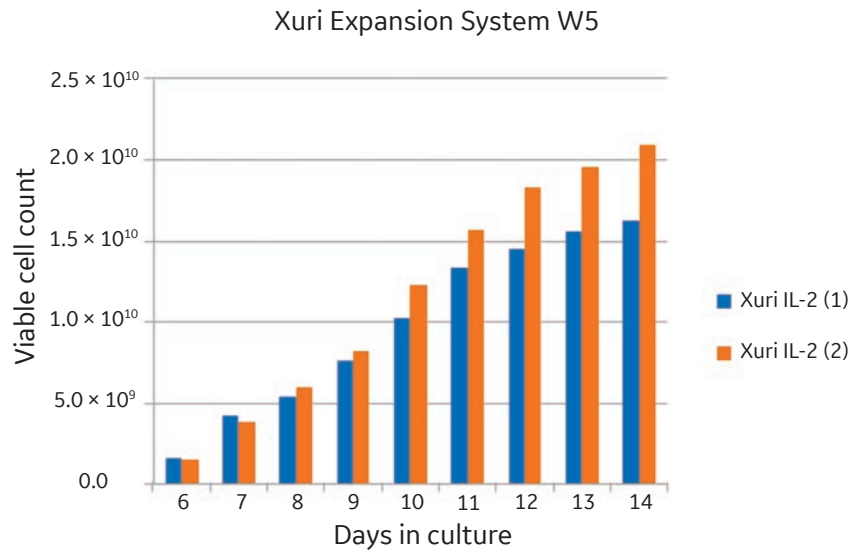


Figure 5.4: Viable cell counts of human primary T lymphocytes cultivated with two batches of Xuri IL-2 (1, 2) in Xuri Cell Expansion System W5 over 9 days succeeding static culture for 5 days.

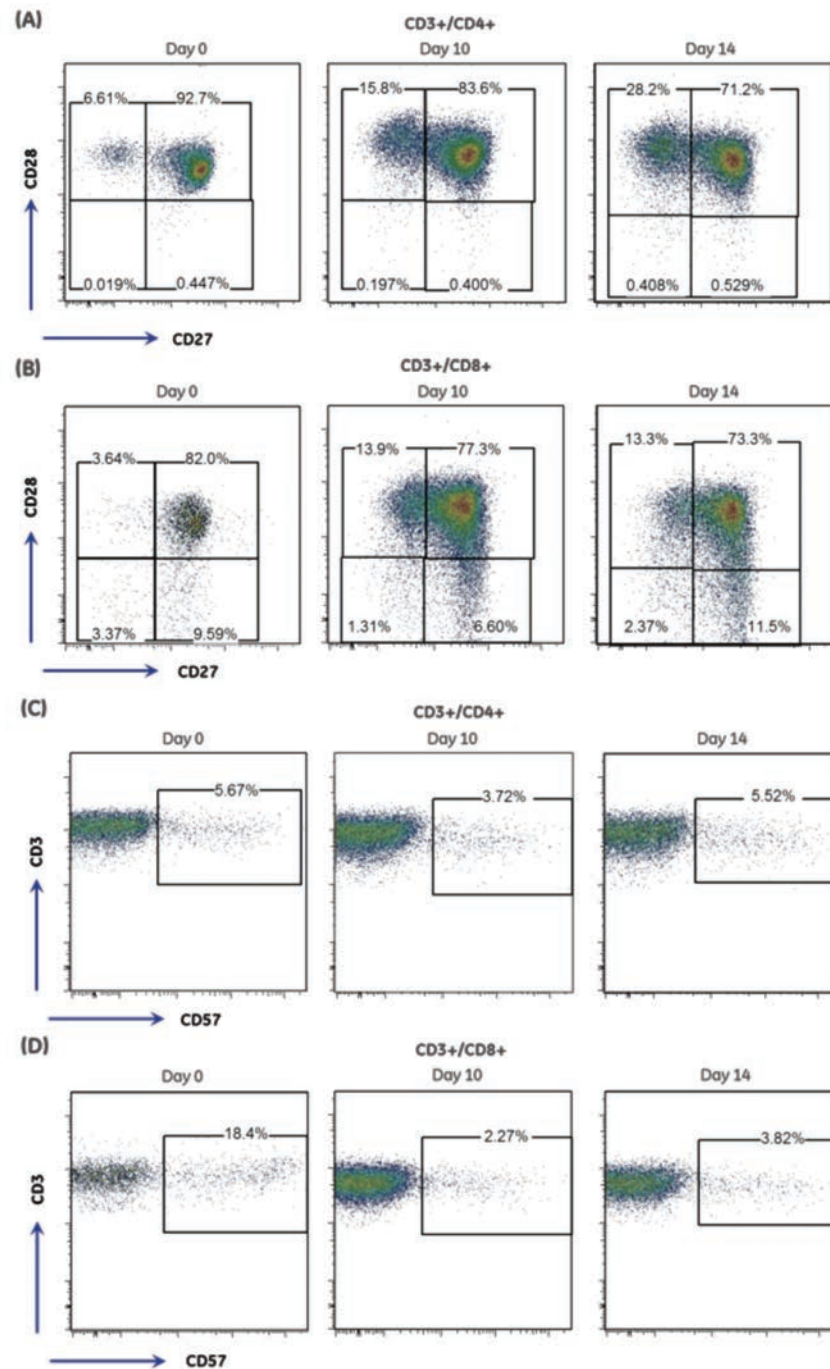


Figure 5.5: Expression of co-stimulatory molecules CD28 and CD27 as well as CD57 in CD3+/CD4+ T (helper) cells and CD3+/CD8+ (cytotoxic) T cells in Xuri Cell Expansion System W5 at day 0, day 10 and day 14 of culture (lymphocytes were gated based on their forward- and side-scatter profile). (A) Percentage CD28+ and CD27+ within CD3+/CD4+ T cells; (B) Percentage CD28+ and CD27+ within CD3+/CD8+ T cells. (C) Percentage CD57+ within CD3+/CD4+ T cells; (D) Percentage CD57+ within CD3+/CD8+ T cells.

Result

Xuri IL-2 supported the proliferation of human T lymphocytes during a 14 days standard expansion procedure. The cumulative cell yields achieved after 5 days in static culture for the two Xuri IL-2 batches were 1.56×10^9 and 1.42×10^9 , and after 9 days in a Xuri W5 expansion system were 1.62×10^{10} and 2.09×10^{10} .

The expression pattern of the cell surface markers CD27 and CD28 was high on both CD4+ (A) and CD8+ (B) T cells, and after 14 days of culture, the majority of T cells were in the early/intermediate stages of differentiation. Flow cytometric analysis showed also no accumulation of senescent cells (CD57+) for CD4+ (C) and CD8+ (D) T cells throughout the 14 day culture period with Xuri IL-2.

5.4 T Lymphocyte viability studies

Requirement

Human T lymphocytes grown with Xuri IL-2 in Xuri Cell Expansion System (Xuri W5) shall maintain a viability of at least 90%.

Experimental set up

Frozen human peripheral blood mononuclear cells were initially cultivated in static cell culture (T225 flasks) with X-VIVO 10 (Lonza) and 5% human serum (heat inactivated) plus 200 IU/mL Xuri IL-2. Cells were concurrently cultured with two batches of Xuri IL-2 and activated using antibody coated beads. Cells were counted from day three on and expanded until they passed the number needed for seeding in a 2 L Cellbag (5×10^8). After 5 days in static culture, cells were transferred and cultivated on a Xuri W5 Cell Expansion System for an additional 9 days under perfusion. Media composition and Xuri IL-2 concentration were kept the same as in static culture. Cell count and viability analysis were performed each day.

Data

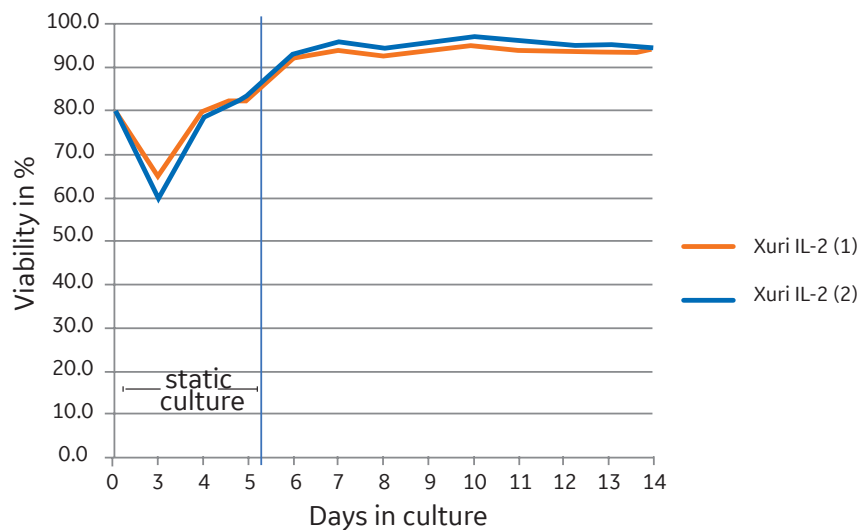


Figure 5.6: Cell viability of human primary T lymphocytes cultivated with two batches of Xuri IL-2 (1, 2) in static and subsequent perfusion culture over 14 days.

5 Validation

5.4 T Lymphocyte viability studies

Result

Cell cultures grown with Xuri IL-2 were able to maintain high viability (>90%) in a Xuri W5 Cell Expansion System.

5.5 Shelf life and product stability

Requirement

Xuri IL-2 shall have a shelf life of at least 18 months after manufacturing. The biological activity shall be comparable to the biological activity of product 24 months after production.

Experimental set up

Shelf life

To characterize differences between independently produced batches, the following tests were used to determine the product quality in the stability studies according to the QC release criteria (see [Product release criteria, on page 6](#)):

- Visual appearance
- Biological activity using a HT-2 cell proliferation assay
- pH after reconstitution
- Molecular weight by SDS-PAGE analysis with Coomassie blue staining
- Purity by reducing and non-reducing SDS-PAGE with silver staining, as well as RP-HPLC
- Endotoxin by LAL test according to EP and USP
- Protein concentration by Lowry method

Two batches of the product were tested directly after production as well as 12 months and 24 months after production stored at the recommended temperature of 2 to 8°C. An additional batch was tested directly after production as well as 12 months later.

Product Stability

To demonstrate the stability of the product, the biological activity of two batches of Xuri IL-2 was determined following incubation of unopened vials in a stability chamber at 30°C ($\pm 2^\circ\text{C}$) with a relative humidity of 75% ($\pm 5\%$) for 7 days. The biological activity is expected to vary no more than 30% compared to the original value at day 0.

5 Validation

5.5 Shelf life and product stability

Test	Specification	Directly after production			12 months post production			24 months post production	
		Batch 1	Batch 2	Batch 3	Batch 1	Batch 2	Batch 3	Batch 1	Batch 2
Visual appearance	White powder/ no foreign particles	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass
Biological activity	12.6–23.4 MIU/mL	17.5 MIU/mL	20.8 MIU/mL	21.1 MUI/mL	18.1 MIU/mL	19.2 MIU/mL	16.0 MIU/mL	16.2 MIU/mL	18.9 MIU/mL
pH	7.0–8.0	7.6	7.7	7.0	7.3	7.3	7.1	7.5	7.4
Mol. weight	14 kDa–16 kDa	14.9 kDa	15.2 kDa	14.4 kDa	15.2 kDa	15.3 kDa	14.3 kDa	15.2 kDa	15.2 kDa
Reducing SDS-PAGE	Purity >97%	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass
Non-reducing SDS-PAGE	Purity >97%	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass
Endotoxin	<25 EU/mg	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass
Protein Concentration	0.9–1.1 mg/mL	1.1 mg/mL	1.1 mg/mL	1.1 mg/mL	1.1 mg/mL	1.1 mg/mL	1.1 mg/mL	1.0 mg/mL	1.1 mg/mL

Figure 5.7: Shelf Life

Batch	Biological Activity on Day 0	Biological Activity after 30°C for 7 days	70% to 130% of the Day 0 Biological Activity
Xuri IL-2 (1)	21.1 MIU/mL	17.98 MIU/mL	14.8–27.4 MIU/mL
Xuri IL-2 (2)	20.8 MIU/mL	19.62 MIU/mL	14.6–27.0 MIU/mL

Figure 5.8: Product Stability

Result

Shelf life

All batches tested fulfil the specification for all time points measured. A shelf life for 18 months applies with a proof of passing the criteria at a time point after that (24 months).

Product stability

After 7 days of incubation at 30°C, both batches passed specification as biological activity was determined to be within the acceptable limits (70–130% of the Day 0 biological activity).

5.6 Batch to batch validation

Requirement

Xuri IL-2 shall support reproducible proliferation of HT-2 cells and activated human T lymphocytes independent of production batch.

Experimental set up

HT-2 cells

HT-2 cells (obtained from ATCC as HT-2 clone A5E) were cultured in triplicate in T25 flasks and maintained in medium containing 200 IU/mL Xuri IL-2 for 7 days. Fresh medium containing 200 IU/mL Xuri IL-2 was added on a daily basis to keep the cell concentration at $3 \text{ to } 9 \times 10^5$ cells/mL and cells were passaged once before day 7. Samples were taken to determine the cell numbers on a daily basis. Cell counts were performed using a Nucleocounter™ following the manufacturer's instructions. Batch variability was judged by cell count comparison between the two batches on days 3, 5 and 7. Cell counts were determined as the mean viable number and % viability across the three replicate flasks.

T lymphocytes

Frozen human peripheral blood mononuclear cells were initially cultivated in static cell culture (T225 flasks) with X-VIVO 10 (Lonza) and 5% human serum (heat inactivated) plus two batches of 200 IU/mL Xuri IL-2. Cells were activated using antibody coated beads. Cells were counted from day three on and expanded until they passed the number needed for seeding in a 2 L Cellbag (5×10^8). After 5 days in static culture, cells were transferred and cultivated on a Xuri W5 Cell Expansion System for an additional 9 days under perfusion. Media composition and Xuri IL-2 concentration were kept the same as in static culture. Batch variability was judged by cell count comparison between the two batches on days 3, 4 and 5 in static culture and days 8, 10 and 14 in Xuri Expansion System.

Data

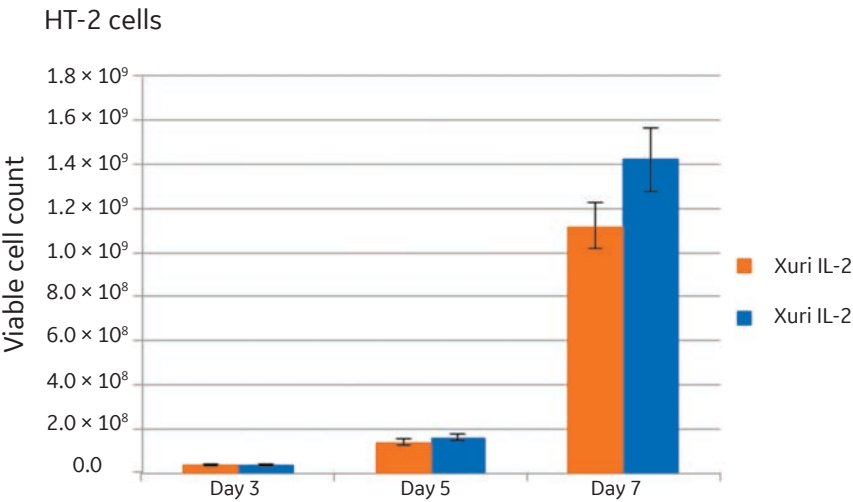


Figure 5.9: Comparison of viable cell count of HT-2 cells cultivated with two different batches of Xuri IL-2 (1, 2) over 7 days. Error bars represent the standard deviation (STDev) of the mean of viable cell counts from three flasks.

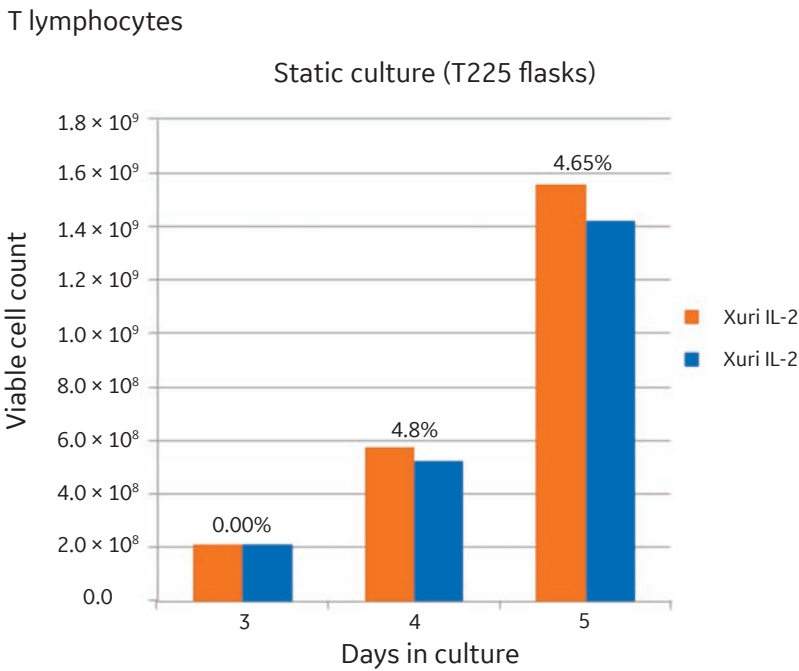


Figure 5.10: Comparison of viable cell count of human primary T lymphocytes cultivated with two different batches of Xuri IL-2 (1, 2) in static T225 culture over 5 days.

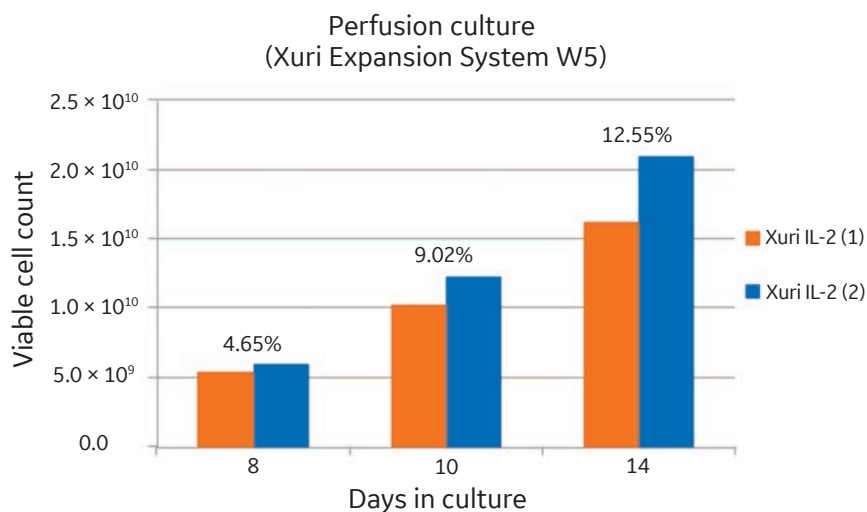


Figure 5.11: Comparison of viable cell count of human primary T lymphocytes cultivated with two different batches of Xuri IL-2 (1, 2) under perfusion in Xuri Cell Cultivation System W5 over 9 days.

Result

HT-2 cells

A significant difference in cumulative cell counts between batches is earliest detectable at day 7 ($P < 0.05$) as shown by one-way analysis of variance (ANOVA).

T lymphocytes

In static culture of T lymphocytes the highest variability detected was 4.8%. In Xuri Expansion System the variability increases over time reaching 9.0% at day 10 and a measured maximum of 12.6% on day 14.

6 Shipment

Products were shipped on blue ice and validated by data logging to determine whether the desired temperature range of 2–8°C is maintained throughout the transportation process. Short periods of time (several hours) where the shipment may be held at greater than 8°C but less than 30°C are acceptable as stability at 30°C has been demonstrated for 7 days with biological activity within the acceptable range. Temperature deviations below the minimum of 2°C during shipment for short periods are considered minimal as lyophilized proteins in general show little sensitivity to temperatures below 0°C.

To be within the validated specifications and the limitations of the product stability the transfer of the product between warehouses or from warehouse to the end user lies within 5 days.

7 Appendices

Appendix A: Product documentation references

For the following product specific documents please refer to: [cytiva.com/certificates](https://www.cytiva.com/certificates)

Certificate of analysis (C of A)

Xuri IL-2 (10 µg):	29082698
Xuri IL-2 (1 mg):	29082699

Further information on the products can be found at: [cytiva.com](https://www.cytiva.com)

Instructions for use (IFU)

Xuri IL-2:	29083091
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Material safety data sheet (MSDS)

Xuri IL-2:	29083903
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Appendix B: External references

EP	European Pharmacopeia
USP	United States Pharmacopeia
ISO 13408-2	Aseptic processing of health care products -- Part 2: Filtration
ISO 13408-3	Aseptic processing of health care products -- Part 3: Lyophilization
ISO 9001	Quality Management System Requirements
USP<1043>	Ancillary Materials for Cell-, Gene-, and Tissue- Engineered Products

Appay V, *et al* Memory CD8+ T cells vary in differentiation phenotype in different persistent virus infections. *Nat Med* 8(4): 379-385 (2002)

Brenchley JM, *et al* Expression of CD57 defines replicative senescence and antigen-induced apoptotic death of CD8+ T cells. *Blood* 101 (7): 2711-2720 (2003)

Appendix C: Terms and abbreviations

SDS-PAGE:	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
ELISA:	Enzyme-linked immunosorbent assay
QMS:	Quality Management System
ATCC:	American Type Culture Collection
RP-HPLC:	Reverse-phase high-performance liquid chromatography
LAL test:	Limulus amebocyte lysate test
ANOVA:	Analysis of Variance
cGMP:	Current Good Manufacturing Practice

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