| | |
|---|---|
| **SOP Title:** | **CTLL-2 Proliferation Assay for Determination of Bioactivity of Recombinant Human Interleukin 2 (rhIL-2)** |
| **SOP Number:** | **16155** |
| **Revision:** | **01** |

## TABLE OF CONTENTS



## 1. PURPOSE

This procedure describes the assay for determining the bioactivity of recombinant human interleukin 2 (rHuIL-2) using CTLL-2 cell proliferation.

## 2. SCOPE

This procedure applies to Biopharmaceutical Development Program (BDP) personnel performing this CTLL-2 cell-based assay.

## 3. RESPONSIBILITIES

### 3.1 Director, Process Analytics/Quality Control (PA/QC)

- Defines this procedure.
- Trains personnel.

### 3.2 Process Analytics/Quality Control (PA/QC)

- Performs this procedure.
- Reviews the data generated by this procedure.

### 3.3 Quality Assurance

- Provides quality oversight.

## 4. MATERIALS AND REAGENTS

| Part Number | Description | BDP Approved Substitution Permitted? |
|---|---|---|
| 10089 | RPMI 1640 medium (with L- Glutamine) | ☒ YES ☐ NO |
| 10109 | Heat-inactivated FBS | ☒ YES ☐ NO |
| 30767 | Recombinant Human Interleukin-2 (rhIL-2) | ☒ YES ☐ NO |
| 30546 | MTS: Cell titer 96 Aqueous one Solution Cell proliferation Assay | ☐ YES ☒ NO |
| N/A | Type I $H_2O$, In-house preparation from Sartorius Water System | ☒ YES ☐ NO |
| 10095 | Trypan Blue | ☒ YES ☐ NO |
| 30599 | Sterile 0.9% sodium chlorid | ☒ YES ☐ NO |
| 30295 | Sterile Water for Injection (WFI), | ☒ YES ☐ NO |
| 30656 | Hank's buffered salt solution (HBSS) | ☒ YES ☐ NO |
| 20104 | 5 mL disposable pipettes | ☒ YES ☐ NO |
| 20100 | 10 mL disposable pipettes | ☒ YES ☐ NO |
| 20102 | 25 mL disposable pipettes | ☒ YES ☐ NO |
| 20738 | 10 uL pipette tips | ☒ YES ☐ NO |
| 21767 | 250 uL pipette tips | ☒ YES ☐ NO |
| 20769 | 1000 uL pipette tips | ☒ YES ☐ NO |
| 20481 | 50 mL reagent reservoir | ☒ YES ☐ NO |
| 20659 | 1.5 mL Eppendorf tube | ☒ YES ☐ NO |
| 20007 | Cryovials tubes | ☒ YES ☐ NO |

| Part Number | Description | BDP Approved Substitution Permitted? |
|---|---|---|
| 20184 | Filter (0.2 um) | ☒ YES ☐ NO |
| 21611 | T-25 cell culture flask | ☒ YES ☐ NO |
| 21610 | T-75 cell culture flask | ☒ YES ☐ NO |
| 21603 | T-150 cell culture flask | ☒ YES ☐ NO |
| 20006 | 15 mL disposable centrifuge tube | ☒ YES ☐ N |
| 20140 | 50 mL disposable centrifuge tube | ☒ YES ☐ N |

## 5. EQUIPMENT

- CO2 Incubator
- Biological safety cabinet
- Freezer -20°C
- Hemocytometer
- Microscope
- ELISA Plate Reader (SPECTRA MAX190 from Molecular Devices or other appropriate ones) and Software (Softmax Pro or other appropriate)
- Pipetmen 0.5-10ul, 2-20ul,10-100ul,20-200ul and 100-1000ul (Rainin or VWR, or equivalent)

## 6. CELLS AND CELL CULTURE

6.1 Perform all steps aseptically in a certified BSC, **SOP 19102, Routine Use and Disinfection of Biological Safety Cabinets, Incubators, Shakers, Centrifuges and Bioreactors.** For cell recovery, propagation, medium preparation and documentation, follow S**OP 22140, Mammalian Cell Culture-Initiation and Maintenance of Cell Culture in BQC**. For cell count and cell viability, follow **SOP 13214, Cell Enumeration Techniques for Mammalian Cell Culture.** The volume of medium, samples, or reagents should be within ±5% of indicated volume as below unless specified.

6.2 Cell Line CTLL-2, ATCC #TIB-214 (T lymphocyte; mouse)

6.3 CTLL-2 Cell Culture Medium

| Reagent | Amount |
|---|---|
| RPMI 1640 medium | 450 mL |
| Heat-inactivated FBS (5.2) | 50 mL |
| IL-2 (5.3) | 200 U/mL |

6.4 Label the complete medium as CTLL-2 Cell Culture Medium with the lot number, date prepared, initials, and expiry date based on the component with the shortest expiration date.

6.5 Preparation of CTLL-2 cell assay medium. Supplement 450 mL RPMI 1640 medium with 50mL FBS and Store at 2-8ºC.

6.6 Cell Recovery and passage. Follow **SOP 13209 Mammalian Cell Culture-Initiation and Maintenance of Cell Culture**.

6.7 Cell count and cell viability as per **SOP 13214-Cell Enumeration Techniques for Mammalian Cell Culture**.

## 7. ASSAY PROCEDURE

7.1 Perform cell count and cell viability using Trypan Blue following step 6.7. Cell viability must be ≥90%.

7.2 Wash the cells two times with the initial volume of HBSS (1000rpm, 10min) and adjust the cell concentration to a density of $5x10^5$ cells/mL (viable cells) using assay medium. Then incubate them for 4±0.5hrs in a CO2 incubator at 37°C.

7.3 Preparation of IL-2 Reference

7.3.1 Reconstitute the vial containing 1,000,000 IU of rhIL-2 with 1 mL sterile 0.9% sodium chloride or sterile water for injection (WFI). Aliquots are stored at -20°C.

7.3.2 Dilute the IL-2 Reference Standard to an Initial dilution (DF1) of 10,000 IU/mL.

7.3.3 Calculate the volume of dilution buffer (Vs) as:

7.3.4 Vs = [Concentration of Reference Standard (IU/mL) × 10 µL ÷ 10,000 IU/mL] -10 µL

7.3.5 Take 10 µL of Reference Standard and Vs µL of Assay Medium. Mix well and label as DF1.

7.3.6 Dilute the DF1 to the second dilution of 400 IU/mL: Take 40 µL of the DF1 and 960 µL of Assay medium, mix well and label as Sta01.

7.4 Preparation of Test Samples

Sample dilution is dependent on protein concentration of the sample. The starting dilution for test sample is ~ 40 ng/mL and may be labeled as sample01. A titration curve covering the entire standard IL-2 range may be generated from 400 IU/ml to ~0.002 IU/ml.

7.5 The Reference and Test Samples can be run in series dilution (1:3) in an empty plate as described below. Alternative way for dilution can be also run and need to be recorded in detail.

7.5.1 Using a 50-300 multiple-channel pipettes, add the assay medium into the columns 1-10 of the plate at 100µL/well.

7.5.2 Using a 50-300 multiple-channel pipettes, add the Starting Std01 or sample01 into the column 11 at 150µL/well in triplicate.

7.5.3 Using a 5-50 multiple-channel pipettes, take 50µL/well from the column 11 into the column 10 in triplicate and mix well by pipetting up and down 5 times.

7.5.4 Using a 5-50 multiple-channel pipettes, take 50µL/well from the column 10 into the column 9 in triplicate and mix well by pipetting up and down 5 times.

7.5.5 Using a 5-50 multiple-channel pipettes, take 50µL/well from the column 9 into the column 8 in triplicate and mix well by pipetting up and down 5 times.

7.5.6 Using a 5-50 multiple-channel pipettes, take 50µL/well from the column 8 into the column 7 in triplicate and mix well by pipetting up and down 5 times.

7.5.7 Using a 5-50 multiple-channel pipettes, take 50µL/well from the column 7 into the column 6 in triplicate and mix well by pipetting up and down 5 times.

7.5.8 Using a 5-50 multiple-channel pipettes, take 50µL/well from the column 6 into the column 5 in triplicate and mix well by pipetting up and down 5 times.

7.5.9 Using a 5-50 multiple-channel pipettes, take 50µL/well from the column 5 into the column 4 in triplicate and mix well by pipetting up and down 5 times.

7.5.10 Using a 5-50 multiple-channel pipettes, take 50µL/well from the column 4 into the column 3 in triplicate and mix well by pipetting up and down 5 times.

7.5.11 Using a 5-50 multiple-channel pipettes, take 50µL/well from the column 3 into the column 2 in triplicate and mix well by pipetting up and down 5 times.

7.5.12 Using a 5-50 multiple-channel pipettes, take 50µL/well from the column 2 into the column 1 in triplicate and mix well by pipetting up and down 5 times and pipette out 100µL/well sample to waste.

7.6 After the incubation, transfer cell suspension (100µL/well) into the wells of the above plate containing IL-2.

7.7 Keep three wells containing cells without test articles as controls for each Samples and keep at least three wells containing assay medium only as blank.

7.8 Incubate above plate in a $CO_2$ incubator at 37ºC for 44±2hrs.

7.9 Using a multiple-channel pipettes, add 20 µl/well of MTS solution (5.4) and

7.10 Incubate the plate for an additional 4±0.5 hours at 37ºC in a $CO_2$ incubator.

7.11 Read the plate using a microplate at 490nm. Follow SOP 16144, Operation of SpectraMax Series (190, 384 plus, M2, M5e, etc.) Plate Readers from Molecular Devices.

7.12 Print out the raw data and template and attach to the QC Test Request form. Initial, date and label printout with the QC test request number.

7.13 Record use, preventative maintenance, and standardization of the instrument in the equipment Logbook as per **SOP 21531 - Equipment Logs.**

7.14 Generate and maintain all documentation relevant to this SOP according to SOP **21409 - Good Documentation Practices.**

## 8. DATA ANALYSIS

8.1 Data analysis is performed as described in the Instruction Manual of the instrument supplier and **SOP 16144, Operation of SpectraMax Series (190, 384 plus, M2, M5e, etc.)** Plate Readers from Molecular Devices. For estimation of test sample titer relative to that of the reference standard, the reference sample block is labeled as standards and the protein values are entered in the template for the standard series. Test sample blocks are labeled as unknown dilution factors and entered in the template. The standard curve is plotted, and the curve fit is selected both for Graphing and the standard curve calculation. A 4-parameter fit analysis is performed using a standard curve constructed with concentration on the X-axis and Mean ODs on the Y-axis with error (Std Dev). Alternate modes of analysis may be performed and explained if required.

8.2 When the activity of test samples is to be calculated from the titration curve, use only the test sample dilutions whose OD readings fall well within the smooth central region of the 4-parameter curve (Do not use sample dilutions whose OD readings are in the plateau or apex of the curve).

8.3 The following criteria should be met for the assay acceptance or validity:

8.3.1 The assay signal response is the ratio of the highest average value on the Reference Standard curve over the lowest average value from the Reference Standard curve. The assay signal response must be ≥ 5.

8.3.2 The CV% of OD readouts for any set of replicate dilutions in the range used for calculation (the steep or smooth region of the 4-parameter curve) should be within 25%.

8.3.3 R2 for curve fit should be ≥ 0.985.

8.4 Bioactivity of test articles will be calculated by the ED50 ratio and back-calculation modes and reported as specific activity.

## 9. DOCUMENTATION AND RECORDS

9.1 Generate and maintain all documentation relevant to this SOP according to S**OP 21409, Good Documentation Practices.**

9.2 All protocols, raw data, computer records, completed **Forms 16155-01 and 16155-02**, and the original copy of the final report are attached to the QC Test Request (QCTR) and submitted for review and approval.

9.3 The approved QCTR is maintained by BQA Documentation as per **SOP 21407, Record Retention. Documentation**.

## 10. REFERENCES AND RELATED DOCUMENTS

| Document Number | Title |
|---|---|
| 13209 | Mammalian Cell Culture-Initiation and Maintenance of Cell culture |
| 13214 | Using a Hemocytometer to Determine Density, Viability, Generation Time and Doubling Time for Mammalian Cells |
| 16144 | Operation of SpectraMax Series (190, 384 plus, M2, M5e, etc.) Plate Readers from Molecular Devices. |
| 16155-01 | Measurement of IL-2 Bioactivity using CTLL-2 Cell Proliferation Assay |
| 16155-02 | Reagents, Materials and Equipment |
| 19102 | Routine Use and Disinfection of Biological Safety Cabinets, Incubators, Shakers, Centrifuges |
| 21407 | Record Retention |
| 21409 | Good Documentation Practices |
| 21531 | Equipment Logs |
| 22140 | Mammalian Cell Culture-Initiation and Maintenance of Cell Culture in BQC |