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1.0 Purpose

The purpose of this assay is to determine the bioactivity of recombinant human interleukin 15 (rHuIL-15) by using CTLL-2 cell proliferation assay.

2.0 Scope

This SOP is to be used for qualitative and quantitative measurement of the CTLL2 cell proliferation by IL-15 and to compare bioactivity of different lots of products relative to a reference lot and to monitor stability of IL15.

3.0 Authority and Responsibility

3.1 The Director, Process Analytics/Quality Control (PA/QC) has the authority to define this procedure.

3.2 The Director, PA/QC is responsible for training laboratory personnel and documenting this training to Biopharmaceutical Quality Assurance (BQA).

3.3 PA/QC personnel are responsible for the performance of this procedure.

3.4 PA/QC personnel are responsible for reviewing the data and documentation of the results of this procedure.

3.5 BQA is responsible for quality oversight of this procedure.

4.0 Equipment and Accessories Required

4.1 CO2 Incubator, Forma Scientific, Inc., or equivalent.

4.2 Biological safety cabinet, Baker (Model B6-0001), or equivalent.

4.3 Water bath

4.4 Hemocytometer

4.5 Microscope

4.6 ELISA Plate Reader (SPECTRA MAX190 from Molecular Devices or equivalent) and Software (Softmax Pro or equivalent).

4.7 96 well cell culture cluster, BDP PN 20050, or BDP approved equivalent.

4.8 Pipetman 0.5-10 µL, 2-20 µL, 10-100 µL, 20-200 µL and 100-1000 µL, (Rainin or VWR, or equivalent).

4.9 Multi-channel pipettes (8 and 12 channels).

4.10 Disposable pipettes 5 mL (BDP PN 20104), 10 mL (BDP PN 20100), 25 mL (BDP PN 20102), or BDP approved equivalent.

4.11 Pipet Tips 10 µL (BDP PN 20738), 250 µL (BDP PN 21767), 1000 µL (BDP PN), or BDP approved equivalent.

4.12 Reagent Reservoir, 50 mL, BDP PN 20481, or BDP approved equivalent.

4.13 Eppendorf tube, 1.5 mL (BDP PN 20659), Cryovial tubes (BDP PN 20007), or BDP approved equivalent.

4.14 Filter (0.2 µm), BDP PN 20184, or BDP approved equivalent.

4.15 Cell culture flask, T-25 (BDP PN 21611), T-75 (BDP PN 21610), T150 (BDP PN21603) or BDP approved equivalent.

4.16 Disposable centrifuge tubes, 15 mL (BDP PN 20006), 50 mL (BDP PN 20140), or BDP approved equivalent.

4.17 Centrifuge, Beckman Coulter or equivalent.

4.18 Refrigerator (2-8°C), Thermo Electron or equivalent.

4.19 Freezer (-20°C), Revco or equivalent.

4.20 Freezer (-65°C or below), Thermo Electron or equivalent.

4.21 Plate shaker.

5.0 Reagents

5.1 RPMI 1640 medium (with L- Glutamine), BDP PN 10089, or BDP approved equivalent.

5.2 Heat-inactivated FBS, BDP PN 10109, or BDP approved equivalent.

5.3 Interleukin-2 (IL-2) for tissue culture, BDP PN 30767 or BDP approved equivalent.

5.4 MTS: Cell titer 96 Aqueous One Solution Cell Proliferation Assay, BDP PN 30546 or BDP approved equivalent.

5.5 Milli-Q H2O, In-house preparation.

5.6 Trypan Blue, BDP PN 10095, or BDP approved equivalent.

5.7 PBS, BDP PN 30007, or BDP approved equivalent.

5.8 Hank’s buffered salt solution (HBSS), BDP PN 30656, or BDP approved equivalent.

5.9 IL-15 Reference Standard with assigned potency (IU/mg).

5.10 SDS solution (10%), BDP PN 30532, or BDP approved equivalent.

6.0 Cells and Cell Culture

NOTE: Perform all steps aseptically in a certified BSC, follow ***SOP 22909 - Use, Cleaning and Disinfection of Equipment and Laboratories in PA/QC***. For cell recovery, propagation, medium preparation and documentation, follow ***SOP 22140 - Mammalian Cell Culture - Initiation and Maintenance of Cell Cultures in Process Analytics/Quality Control***. The volume of medium, samples, or reagents should be within ± 5% of indicated volume as below unless specified.

6.1 Cell Line CTLL-2, ATCC TIB-214 (T lymphocyte; mouse).

The CTLL-2 cell bank (BDP PN 10510) was generated by the BDP Cell Culture Lab from cells obtained from ATTC. Details of cell bank generation and testing are captured in MPR-C-17 (10-13-06 to 11-2-06) and QC-032875 and QC-032876.

6.2 CTLL-2, Cell **Culture Medium**

Reagent	Amount
RPMI 1640 medium (3.1)	450 mL
Heat-inactivated FBS (3.2)	50 mL
IL-2 (3.3)	200 U/mL

NOTE: IL-2 is added (under sterile conditions) directly into the cell culture flask immediately before use or complete media just before use.

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. Store at 2 - 8ºC.

6.3 Preparation of CTLL-2 Cell **Assay Medium**

Reagent	Amount
RPMI 1640 medium (3.1)	450 mL
Heat-inactivated FBS (3.2)	50 mL

Label as CTLL-2 Cell Assay Medium with the lot number, date prepared, initials, and expiry date based on the component with the shortest expiration date. Store at 2 - 8ºC.

6.4 Cell Recovery and Passage

Follow ***SOP 13209 - Mammalian Cell Culture-Initiation and Maintenance of Cell Cultures***.

6.5 Cell Count and Cell Viability

Follow ***SOP 13214 - Using a Hemocytometer to Determine Density, Viability, Generation Time and Doubling Time for Mammalian Cells***.

7.0 Assay Procedure

7.1 Perform cell count and cell viability following 6.5. Cell viability must be ≥ 95%.

7.2 Wash the cells two times with HBSS (1000 rpm, 10 minutes) and adjust the cell concentration to a density of 5 x 10^5 cells/mL (viable cells) using assay medium. Incubate the cells at 37 ± 2°C in a 5 ± 2% CO_2 and ≥ 70% humidity atmosphere for 4 ± 0.5 hours.

7.3 Preparation of IL-15 Reference and samples.

7.3.1 Dilute the Reference Standard to an initial dilution (DF1) of 10 μg/mL

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

Vs = [Concentration of Reference Standard (μg/mL) × 10 μL ÷ 10 μg/mL]-10 μL

7.3.2 Take 10 μL of Reference Standard and **Vs** μL of PBS. Mix well and label as **DF1**.

7.3.3 Dilute the DF1 to the second dilution of 100 ng/mL: Add 10 μL of the DF1 to 990 μL of assay medium, mix well and label as **DF2**.

7.3.4 Dilute the DF2 to the third dilution of 2 ng/mL:Add 40 μL of the DF2 to 1960 μL of assay medium, mix well and label as **DF3** or **Sta01**.

7.4 Preparation of Test Samples

Sample dilution is dependent on protein concentration of the sample. The starting dilution may be labeled as sample01. A titration curve covering the entire standard IL-15 range may be generated from 2 ng/mL to 0.031 ng/mL.

7.5 The Reference Standard and samples can be run in series dilution (1:2) in an empty plate as described below.

7.5.1 Using a 50-300 multiple-channel pipettes, add the assay medium into the rows B-H of the plate at 100 μL/well.

7.5.2 Using a 50-300 multiple-channel pipettes, add the Starting Std01 or sample 01 into the row A at 200 μL/well in triplicate.

7.5.3 Using a 50-300 multiple-channel pipettes, take 100 μL/well from the row A into the row B in triplicate and mix well by pipetting up and down 5 times.

7.5.4 Using a 50-300 multiple-channel pipettes, take 100 μL/well from the row B into the row C in triplicate and mix well by pipetting up and down 5 times.

7.5.5 Using a 50-300 multiple-channel pipettes, take 100 μL/well from the row C into the row D in triplicate and mix well by pipetting up and down 5 times.

7.5.6 Using a 50-300 multiple-channel pipettes, take 100 μL/well from the row D into the row E in triplicate and mix well by pipetting up and down 5 times.

7.5.7 Using a 50-300 multiple-channel pipettes, take 100 μL/well from the row E into the row F in triplicate and mix well by pipetting up and down 5 times.

7.5.8 Using a 50-300 multiple-channel pipettes, take 100 μL/well from the row F into the row G in triplicate and mix well by pipetting up and down 5 times and pipette out 100 μL/well sample to waste.

7.5.9 Take 200 μL of sta01 or sample01 (2 ng/mL) and 800 μL of Assay Medium, mix well (0.4 ng/mL) and then add 100 μL/well to the plate in triplicate using a 50-300 multiple-channel pipettes.

7.5.10 Take 100 µL of sta01 or sample01 (2 ng/mL) and 900 µL of Assay Medium, mix well (0.2 ng/mL) and then add 100 µL/well to the plate in triplicate using a 50-300 multiple-channel pipettes.

NOTE: Alternatively, the Reference Standard and samples can be run in series dilution (1:2) from column to column in an empty plate. Document the actual dilution details and template in data printout.

7.6 After the 4 ± 0.5 h incubation of the cells in step 7.2, transfer cell suspension into the wells of the plate containing IL-15, 100 µL/well.

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

7.8 Incubate above plate in an incubator at 37 ± 2°C in a 5 ± 2% CO2 and ≥ 70% humidity air atmospheres for 48 ± 2 hours.

7.9 Using a multiple-channel pipettes, add 20 µL/well of MTS solution (5.4) and incubate the plate for an additional 4 ± 0.5 hours at 37ºC in a CO2 incubator.

7.10 Read the plate at 490 nm using a microplate reader as per the Operating Procedure for the Operation of ELISA Plate Reader, (Molecular Device Instruction Manual for Softmax 190 and Softmax 384 Plus).

7.11 Add 10% SDS solution (5.10) 25 µL/well and shake for 1 minute on a titer plate shaker at room temperature, discard the plate.

7.12 Print out the raw data and template and attach them to the QC Test Request or date file. Initial, date, label with QC Test Request number.

8.0 Data Analysis and Acceptance Criteria

8.1 Data analysis is performed using the 4-parameter curve fit as described in the Instruction Manual of the instrument supplier. For estimation of test sample titer relative to that of the reference standard, the reference sample block is labeled standards and the protein values are entered in the template for standard series. Test sample blocks are labeled unknown dilution factors and entered in the template. Standard curve is plotted and the curve fit is selected both for Graph and the standard curve. A 4-parameter fit analysis is performed using standard curve constructed with concentration on X-axis and Mean ODs at Y-axis with error Stdev. Alternate modes of analysis may be performed and explained if required.

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

8.2.1 Background OD (media only) should be ≤ 0.5.

8.2.2 The assay signal 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 must be ≥ 5.

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

8.2.4 R2 for curve fit should be ≥ 0.985.

8.2.5 If these criteria are not met, discuss with the Supervisor.

8.3 Bioactivity of test articles will be calculated by ED_{50} ratio and back-calculation mode and expressed in Units/mg under the assay conditions as well as IU/mg based on standard curve generated using rhIL15 Reference Lot with an assigned activity value (IU/mg).

8.3.1 For ED_{50} ratio-calculation mode, the activity of test samples will be calculated using the formula: (ED_{50} of reference lot ÷ ED_{50} of test article) × Activity of Reference Standard (IU/mg). Document calculations on data printout.

8.3.2 For back-calculation mode, the activity of test samples will be calculated from the OD read-out of known dilutions by extrapolation to the 4-Parameter curve fit standard curve generated from Reference Lot (convert concentration from ng/mL to IU/mL on X-axis based on the assigned activity value of Reference Lot (IU/mg)). The test article will be treated as sample of unknown concentration and activity extrapolation will be based on the absorbance reading of test articles extrapolated to standard curve. Values adjusted for sample dilutions for the samples with absorbance in the steep part of the standard curve absorbance reading will be averaged (Do not use sample dilutions whose OD readings are in the plateau or apex. There should be at least three dilutions of test article with OD values in the steep part of the standard curve) and reported as the activity of the test article. Document calculations on data printout.

9.0 Documentation

9.1 All protocols, raw data, computer records, completed **Forms 16133-01** and **16133-02**, and the original copy of the final report will be maintained by BQA Documentation.

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

10.0 References and Related Documents

10.1 Operating Procedure for the operation of ELISA Plate Reader (Molecular Device Instruction Manual for Softmax190 and Softmax 384 plus).

10.2 **SOP 13209** *Mammalian Cell Culture-Initiation and Maintenance of Cell Culture.*

10.3 **SOP 13214** *Using a Hemocytometer to Determine Density, Viability, Generation Time and Doubling Time for Mammalian Cells.*

10.4 **SOP 21409** *Good Documentation Practices.*

10.5 **SOP 22140** *Mammalian Cell Culture - Initiation and Maintenance of Cell Cultures in Process Analytics/Quality Control.*

10.6 **SOP 22909** *Use, Cleaning and Disinfection of Equipment and Laboratories in PA/QC.*

11.0 Change Summary