MPS-015
PHENIX – LIPIDTOX IMAGE ANALYSIS

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University of Pittsburgh Drug Discovery Institute

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Revision history			
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1.0	Dillon Gavlock	March 14, 2025	Original Document

TABLE OF CONTENTS

A. PURPOSE AND APPLICABILITY

This protocol outlines the steps necessary to complete LipidTox analysis on the PerkinElmer Opera Phenix. This protocol will take you through how to get started quickly using the preconfigured analysis created for our standard models (LAMPS, vLAMPS, bpLAMPS) and how to adjust settings in Harmony for your specific image condition. This protocol assumes you acquired images using the model specific Phenix Imaging protocol.

B. SUMMARY OF METHOD

C. DEFINITIONS

Term	Explanation

D. QUALIFICATIONS

1. Laboratory personnel will read the following documents provided by PerkinElmer:
    a. Opera Phenix User Manual
        i. Section 5.1.0.0 - 5.1.8.2 (Harmony Software User Interface Section)
        ii. Section 5.2.0-5.2.7 (Analysis Building Block Reference)
    b. Opera Phenix Analysis Manual
        i. Section 3.24.0-3.24.4 (Lipid Droplet Analysis)
2. Successfully complete training by Dillon Gavlock.

E. HEALTH AND SAFETY WARNINGS

1. All chemicals must be handled in compliance with the University of Pittsburgh's Chemical Hygiene Plan Safety Manual.
2. All biological material must be handled in compliance with the University of Pittsburgh's Biosafety Guidelines.

F. CAUTIONS

G. INTERFERENCES

H. EQUIPMENT AND SUPPLIES

- Harmony v5.2

I. PROCEDURAL STEPS

1. Turn on the Opera, open Harmony 5.0 and enter your profile information.
    a. If you do not have a profile on the Phenix please talk to Dillon Gavlock about creating one.

2. When you open Harmony, navigate directly to the “Analysis” tab.
3. On the left-hand side you will see the configuration bar that houses all the information for setting up an analysis. You’ll want to load the following analysis protocol:
    a. LipidTox_Analysis_v2
4. In this protocol you will have all the preconfigured settings loaded into the configuration panel. They include the appropriate analysis building blocks to complete the lipid quantification of our model.
5. Make sure to select all images from the stack panel by right clicking and selecting “Select All”
6. Be sure that you review each analysis block and that they are quantifying the Cy5 channel (LipidTox)
7. Once you’re ready to complete the analysis, click “Save” and then go to the “Evaluation” tab at the top and select “Start”
8. Once the analysis is completed, export the data to the desired RFE folder
9. Once the data is exported go to the RFE and merge all the desired data into a single excel sheet adding the experimental metadata for each chip/chamber.
10. Create graphs and charts appropriate for your interests, generally we report the “Normalized Area”, “Normalized Intensity”, and “Normalized Number of Lipids”. You can also report their unnormalized values which are present in the analysis files.

J. DATA AND RECORDS MANAGEMENT

K. QUALITY ASSURANCE AND QUALITY CONTROL

L. REFERENCES