

# Application for Cytotoxicity Assays at Keck-UWCCC Small Molecule Screening Facility

Updated 11-29-2005 NRP

The Small Molecule Screening Facility (SMSF) is a core facility that is required to operate on a chargeback mechanism. All users of the facility will be charged for the laboratory consumables, reagents and staff time. Estimates of these charges can be given after a protocol is agreed upon. So we may better serve you, please fill out the following information.

## **Description of Cytotoxicity Services**

### **Cell Lines:**

The SMSF cytotoxicity assays are run in the following cell lines-you may choose any or all:

<b>Cell Line</b>	<b>Description</b>
HCT-15	Human colorectal adenocarcinoma
HT-29	Human colorectal adenocarcinoma
MCF-7	Human breast adenocarcinoma
SF-268	Human CNS glioblastoma
SK-OV-3	Human ovary adenocarcinoma
NCI-H460	Human lung carcinoma
A549	Human lung Adenocarcinoma
NCI/ADR-RES	Human breast Adenocarcinoma
Hep3B	Human liver Carcinoma
Du145	Human prostate Carcinoma
HL-60	Human acute promyelocytic leukemia
MDA-MB-231	Human breast adenocarcinoma
MES-SA	Human uterine sarcoma
MES-SA/DX5	Human uterine sarcoma – drug resistant
NmuMG	Mouse mammary normal epithelial

### **Cell line maintenance for Cytotoxicity Assays:**

Each cell line is maintained by a strict protocol at the SMSF to produce reliable results in cytotoxicity assays. Cells are thawed from frozen stocks for each cytotoxicity assay and grown to confluence in triple-floor cell culture flasks or T-75 cell culture flasks. When the cultures are 80% confluent, the cells are plated for the assay. For this reason, 2 weeks are required to start each cytotoxicity assay.

### **Assays:**

#### *Preliminary Assay to determine compounds with Activity:*

For each compound a preliminary assay will be performed in all cell lines in duplicate to determine if the compounds exhibit cytotoxic effects similar to the parent molecule or doxorubicin. The preliminary assay will be performed at 1 concentration that is equal to 10X the observed IC<sub>50</sub> of the parent molecule\* or doxorubicin. All compounds that exhibit greater than 50% inhibition when compared to a DMSO only control will be tested further in the dose response assay.

# Application for Cytotoxicity Assays at Keck-UWCCC Small Molecule Screening Facility

Updated 11-29-2005 NRP

\*If the parent molecule is a compound that has not been tested in the assay at the Small Molecule Screening Facility, a dose response will be performed with the parent molecule to determine the IC<sub>50</sub> in the assays performed at the Keck-UWCCC SMSF

## *Dose response:*

For each compound to be tested a linear dose response of 2 fold dilutions will be performed with 5 test points and 1 "0" control. The dose responses are performed in triplicate. If 2 of the 3 fall into acceptable limits of variability then the 2 points will be used to determine the IC<sub>50</sub>. All stocks delivered to the small molecule screening facility must be at least 100X of the highest concentration to be tested. ***Adequate compound needs to be supplied to perform the pre-screen and the cytotoxicity assay.***

Because of the narrow range of the dose response, the SMSF will estimate pricing for 2 assays per compound to insure that we will be able to determine the IC<sub>50</sub>. ***The SMSF does not guarantee that the IC<sub>50</sub> will be determined, if the compound does not show inhibition at the concentrations agreed upon for testing, the IC<sub>50</sub> will be reported to you as "greater than concentration tested."***

## *Solvent:*

All samples contain 1% solvent. The solvent used at the SMSF is DMSO and the conditions of the assay have been optimized to this concentration of DMSO. ***If a different solvent is to be used, the SMSF cannot guarantee that the results will fall into acceptable limits of variability as optimized at the facility.***

## *Sample Storage and Handling:*

All samples submitted to the small molecule screening facility will be stored at -20 under in a dessicator. Freeze/thaw cycles are minimized by aliquoting large volumes and preparing compound test plates on the day of compound delivery. The plates and opened compound tubes/vials are layered with argon gas before sealing. On the day of the assay compounds are thawed at room temperature in a dessicator and then prepared for the assay. Before addition to cells, the test compounds are diluted 10 fold in cell culture media. This is done to insure adequate mixing of the compound in the cell culture media before addition to the cells. When added to the cells, the compounds are further diluted 10 fold yielding a 100X dilution.

***All samples must be delivered to the SMSF before 12:00 noon to give the staff adequate time to aliquot and prepare the compound test plates. You will need a minimum of 100ul of 100X of the highest concentration to be tested. If additional compound is required the SMSF will alert you.***

# Application for Cytotoxicity Assays at Keck-UWCCC Small Molecule Screening Facility

Updated 11-29-2005 NRP

## *Controls:*

For each cell line, a test plate will be run in order to calculate the % coefficient of variation and Z-score of the assay. The control plate is run with and without a known cytotoxic agent to cancer cells, Doxorubicin.

***If the controls show high variability, the assay will be repeated in that cell line at no charge to the user.***

## *Assay:*

The cytotoxicity assay performed at the small molecule screening facility is a long assay that does not determine the mechanism of cytotoxicity. Cells are plated on day 1 and allowed to attach for 1 hour. Compounds are added and incubated with cells for 72 hours and then washed and stained with calcein AM for viability.

### **Calcein AM staining for Live Cells -Molecular Probes, Inc.:**

Live cells are distinguished by the presence of ubiquitous intracellular activity, determined by the enzymatic conversion of the virtually nonfluorescent cell-permeant calcein AM to the intensely fluorescent calcein. The polyanionic dye is well retained within live cells, producing an intense uniform green fluorescence in live cells. Cat No: C3099.

### **MitoTracker RED- InVitrogen, Inc.:**

MitoTracker Red is a cell-permeant probe that passively diffuses across the cell membrane of living cells and accumulates in active mitochondria.

***The cytotoxicity assays performed at the SMSF do not determine the mechanism of cytotoxicity of the test compounds. Other assay services will soon be available to determine growth inhibition or apoptotic induction by test compounds.***

## Data Analysis:

- The % Inhibition of growth is calculated for each compound tested at each concentration.
- IC50s can be calculated if growth is inhibited more than 50% at the highest concentration tested using linear interpolation and curve fitting using xlfite 4.0 (IDBS)

Upon completion of the assays, you will receive a detailed report of how the assay was performed, your raw data, and all calculations made and summarized.

## **Timeline:**

When the SMSF receives your request for cytotoxicity assays, the cells will be started from frozen stocks. This is done to maintain the cells at low passage number before the assay is performed and causes a 2 week delay. When there are enough cells available to perform your assay, they will be plated and compounds will be added. The assay is read

# Application for Cytotoxicity Assays at Keck-UWCCC Small Molecule Screening Facility

Updated 11-29-2005 NRP

after 72 hrs. The minimum time required to perform cytotoxicity assays is 1 month, and larger numbers of compounds will take more time.

**Please fill out the attached application and provide the SMSF with your compounds.**

# Application for Cytotoxicity Assays at Keck-UWCCC Small Molecule Screening Facility

Updated 11-29-2005 NRP

## **Application:**

*Please return the following application with your compounds for cytotoxicity testing:*

Name

E-mail

Telephone #

Lab Name (Name of PI)

PI's e-mail

Position in the lab (eg. Lab head, postdoc, graduate student)

Fund account

Grant End Date

## **Description of the Compounds to be tested:**

Briefly describe what the compounds are (e.g. hits from a screen, synthesized compounds, etc.)

## **Compounds to be tested:**

Please fill out the attached table below for all compounds

## **Compound delivery:**

Please deliver compounds to the lab with your completed application before 12noon.

Please make sure there is enough compound available to perform all assays you would like done. You will need a minimum of 100ul of 100X of the highest concentration to be tested. If additional compound is required the SMSF will alert you.

Upon completion of the assay any remaining test compound will be returned to you.



# Application for Cytotoxicity Assays at Keck-UWCCC Small Molecule Screening Facility

Updated 11-29-2005 NRP